Final Report

Acute Inhalation Toxicity Study of MWCNT in Fisher 344 rats

Study No. : GT13-00173

November 2014

the way to trust KCL Korea Conformity Laboratories

BioConvergence Technology Laboratory

Statement

Study code : GT13-00173

Title : Acute Inhalation Toxicity Study of MWCNT in Fisher 344 rats

This study has been performed in compliance with the principles of Good Laboratory Practices and test guidelines in following documents.

1. Guideline for the Testing of Chemical Hazards, National Institute of Environment Research (NIER)[Notice No. 2013-2 (revised 9th, Jan., 2013)]
2. OECD Guideline for the Testing of Chemicals No. 403 'Acute Inhalation Toxicity' (Adopted 7th Sep., 2009)

The stated object in the study protocol was achieved and there were no significant deviations from the aforementioned regulations that affected the quality or integrity of the study. Therefore, the justification of all data in this study was confirmed. The information of the test substance was written from the document that the sponsor provided.

Byung-Gil Choi
Study Director
BioConvergence Technology Laboratory

Nov. 19. 2014
Date

Jin-Kyu Lee
Managing Director
BioConvergence Technology Laboratory

Nov. 19. 2014
Date

QUALITY ASSURANCE STATEMENT

Study No. : GT13-00173

Title : Acute Inhalation Toxicity Study of MWCNT in Fisher 344 rats

This study was subject to audit by the independent Quality Assurance Unit of KCL as indicated below. The findings of each audit were reported to the study director and management as prescribed by Standard Operating Procedures.

The final report audit was designed to confirm that as far as can be reasonably established the methods described and results incorporated in the final report accurately reflect the raw data produced during the study.

Audit phases and dates reported to the responsible personnel were as indicated below and these were based upon the audit records.

Phase Inspected	Date	Reports to Study Director	Reports to Management
Study Plan	2013. 04. 19	2013. 04. 19	2013. 04. 22
Storage of Test substance and vehicle	2013. 04. 25	2013. 04. 25	2013. 04. 25
Animal receipt	2013. 04. 25	2013. 04. 25	2013. 04. 25
Preparation of test substance	2013. 04. 30	2013. 04. 30	2013. 04. 30
Animal care and Administration	2013. 04. 30	2013. 04. 30	2013. 04. 30
Clinical sign	2013. 05. 14	2013. 05. 14	2013. 05. 14
Necropsy	2013. 05. 14	2013. 05. 14	2013. 05. 14
Raw data	2013. 08. 05	2013. 08. 05	2013. 08. 05
Final Report	2013. 08. 05	2013. 08. 05	2013. 08. 05

QA director : Song, Kyung Seuk Ph.D. Date 2013. 08. 05
Auditor, Quality Assurance

* signed original

Study Personnel

Principal Investigator	Jae-Hyuck Sung*	**Date**	05 August 2013
Formulation	Jae-Hyuck Sung*	**Date**	05 August 2013
Animal care	Min-Won Baek*	**Date**	05 August 2013
Necropsy & Pathology	Hye-Jin Kim*	**Date**	05 August 2013
Archiving	Hyo-Dong Kim*	**Date**	05 August 2013

* Signed original

Title Acute Inhalation Toxicity Study of MWCNT in Fisher 344 rats

Objective of Study This study is performed to assess the acute inhalation toxicity and lethal dose 50 (LD_{50}) of MWCNT.

Sponsor

Name : Bioconvergence Technology Laboratory
Korea Conformity Laboratories
Client : Jin-Kyu Lee
Address : 7-44, Songdo-dong, Yeonsu-gu, Incheon, 406-840, Korea
Tel. : +82-32-859-4041 Fax : +82-32-858-0020

Testing facility

Name : Bioconvergence Technology Laboratory
Korea Conformity Laboratories
Address : 7-44, Songdo-dong, Yeonsu-gu, Incheon, 406-840, Korea
Tel. : +82-32-859-4050 Fax : +82-32-858-0020

Study Schedule

Animal acquisition	: 25	April	2013
Exposure	: 30	April	2013
Necropsy	: 14	May	2013
Submission of final report	: 05	August	2014

Archiving of study data

1) Archiving period : Least 5 years after the study termination
2) Data : Study protocol, test substance data, animal acquisition data, raw data, final report and GLP documents
3) Storage room
(1) Archive : CD, relevant document

Contents

1. SUMMARY

This study was performed to evaluate acute inhalation toxicity of multi-wall carbon nanotube (MWCNT) using specific pathogen free (SPF) - Fisher 344 (F344) rats with the concentration of 0, 0.17 (low-dose group), 0.52 (middle-dose group) and 0.83 (high-dose group) ㎎/㎥. The rats were exposed to test substance for 6 hours a day in whole body inhalation chamber. Grouping consisted of 5 male and female rats in each group. Test method conformed to OECD Guideline for Testing of Chemicals No. 403 'Acute Inhalation Toxicity' (Adopted May, 2009).

Environment and the concentration of MWCNT in exposure chamber were measured for the duration of exposure time. Mortalities, clinical signs and body weight changes of experimental animals were evaluated during the observation period of 14 days. After termination of study, gross findings for the surviving animals were observed by examining organs in necropsy. The results were as follows,

No toxic signs or mortality were observed relating to the test substance.
There was no significant difference of body weight changes between control and exposure group.
At the end of study, all animals were subjected to necropsy, and no abnormal gross findings were observed in relation to the test substance.

In conclusion, the lethal concentration 50 (LC_{50}) value for the acute inhalation of MWCNT is considered to be > 0.83 ㎎/㎥ of mass concentration in this study.

2. TEST SUBSTANCE AND VEHICLE

1) Test substance (Appendix 1)

(1) Name : MWCNT

(1) Product Name : K-Nanos-100P

(2) CAS No. : No data

(3) Lot No. : No data

(4) Received date : 25 January 2013

(5) Received quantity : 666.89 g (including a container weight)

(6) Appearance : Powder, Black

(7) Purity : Carbon content > 90 %

(8) Storage condition : Ambient room temperature

(9) Stability : Stable under refrigeration

(10) Handling : Stable under refrigeration

(11) Supplier : Kumho petrochemical Co., Ltd.

2) Vehicle

(1) Name : HEPA filtered fresh air

3) Storage and Treatment

The test substance was kept in a storage room (108-2). At completion of the study, the remaining test substance was kept in a storage room (108-3).

4) Formulation of the test solution

The mixture of test substance and fresh air was used after maintain a constant temperature and pressure using the carbon nanotube (CNT) generator

3. MATERIALS AND METHODS

1) Test animals

(1) Species and strains : Specific Pathogen Free(SPF) Fisher 344 rats (F344/N Slc)

(2) Supplier : Central Lab. Animal Inc. (5F Eun-seok B/D, 64, Umyeon-dong, Seocho-gu, Seoul, Korea)

(3) Producer : Japan SLC, Inc. (3371-8 Kotoh-cho, Hamamatsu, Shizuoka Prefecture 431-1103)

(4) Reason for selection of the species

The animals used in this study, F344 rats, have been applied widely in general toxicity tests as a suitable experimental animal for toxicity testing. In addition, sufficient raw data have been accumulated, and such data are

available in interpretation and evaluation of study results.

(5) Date of acquisition : 25 April 2013

(6) Number of animals received : male 22, female 22

(7) Age of animals received : 7 weeks

(8) Body weights on arrival

① male : 106.74 ~ 133.25 g

② female : 94.95 ~ 118.05 g

(9) Quarantine and acclimation

Animal observation performed on date of acquisition. Microorganism test result was obtained from supplier. Acclimation duration was more than 5 days. Only the most healthy animals were used for study after observing general conditions in the acclimation period. (Appendix 2)

(10) Age at the initiation of the exposure : 8 weeks

(11) Body weights at the exposure (mean±S.E)

① male : 148.91±1.62 g

② female : 127.03±1.34 g

(12) Number of animals used : female 20, male 20

(13) Grouping

The animals were stratified randomly by body weight after measuring body weight one day before initiation of exposure.

(14) Identification of individual animals

To distinguish animals, skin marking (blue marking during acclimation and black marking during main study) was used. Cage card was used for each cage and the animal use log was posted at the entrance of animal room with indication of study number, title, duration of use, name of study director, and name of study personnel.

(15) Handling of remaining animal

Remaining animals sacrificed at the planned date.

2) Environmental and Housing Condition (Annex 3)

(1) Animal care room

① Acclimation period : Inhalation toxicity animal room

② Exposure period : Inhalation toxicity room #1

③ Clinical sign observation period : Inhalation toxicity animal room

(2) Range of temperature and humidity

① Acclimation period : 22.0±1.1 ℃ of temperature
48.7±6.7 %RH of relative humidity

② Exposure period : 22.4±1.8 ℃ of temperature

45.9±7.8 %RH of relative humidity

③ Clinical sign observation period : 21.8±0.9 ℃ of temperature,
51.5±4.2 %RH of relative humidity

(3) Lighting cycle : 12 hrs of lighting duration
(lighting up at 8 a.m. ~ lighting out at 8 p.m.)

(4) Lighting intensity

① Acclimation period : 293 Lux

② Exposure period : 276 Lux

③ Clinical sign observation period : 293 Lux

(5) Ambient noise level

① Acclimation period : 47.3 dB

② Exposure period : 58.9 dB

③ Clinical sign observation period : 47.3 dB

(6) Ammonia concentration

① Acclimation period : less than 5 ppm

② Exposure period : less than 5 ppm

③ Clinical sign observation period : less than 5 ppm

(7) Housing

The 5 animals were housed in polycarbonate cage (360 L×215 W×200 H ㎜) during quarantine, acclimation and clinical sign observation period. And the 1 animal was housed in wire 5-mesh cages (750 W×170 D×150 H ㎜) during exposure periods.

(8) Feeds and water

① Feeds

Radiation sterilized, solid laboratory animal feeds (Teklad Certified Irradiated Global 18 % Protein Rodent Diet, Harlan Co. Ltd., USA) were provided *ad libitum*. DooYeol Biotech Co., Ltd. supplied feeds.

② Water

Incheon, Korea municipal tap water purified by reverse osmosis filtering system was provided *ad libitum* using water bottles.

③ Certification

The feed certification which was provided from the supplier and the water certification from national certificated inspection organization were referred to examine contamination (Annex 4, 5).

3) Methods

(1) Exposure method

① Route : Inhalation (whole body)

② Reason : Inhalation is a major route for MWCNT exposure.

③ Exposure frequency and duration : One time, 6 hr exposure.

④ Exposure location : mainly respiratory system

⑤ Justification for dose setting : The maximal mass concentration capacity for the MWCNT generating system was used in this study to generate the high dose exposure. Actual low and middle dose concentrations were determined by the standard high dose concentration dilution process employed in this system.

(2) Dose group and target concentration

Test substance	Group	Mass (㎎/㎥)	Sex	N	Animal No.
MWCNT	Control	0	M	5	1-5
			F	5	21-25
	Low	0.2 ± 30 %	M	5	6-10
			F	5	26-30
	Medium	0.5 ± 30 %	M	5	11-15
			F	5	31-35
	High	1.0 ± 30 %	M	5	16-20
			F	5	36-40

M; Male, F; Female

4) Test items

(1) Measurement of environment inside animal exposure chamber

Temperature, humidity, pressure and air flow was measured automatically using inhalation toxicity monitoring system at main control center for duration of exposure period.

(2) Generating method for MWCNT

MWCNT were generated as shown in Figure 1. It was used that the CNT generator and whole body inhalation toxicity chamber (HCT 5300 HCT, Korea; MAI-088-01).

(3) Distribution of MWCNT in exposure chamber

Mass concentration was measured with the NIOSH method 0500 using the personal air sampler and polyvinylidene fluoride membrane filter.

(4) Transmission Electron Microscopy (TEM) analysis

The TEM sample, which was collected with nanoparticle collector, was requested for analysis of MWCNT shape.

(5) Clinical signs

Clinical observations including general appearance were conducted daily. Clinical signs were checked 6 hours after exposure and then checked

everyday for 14 days. Individual records were maintained for each animal including the type, date and the grade of clinical signs for each animal. Particular attention was directed to observation of changes to the skin and fur, eyes, mucous membranes, respiratory, circulatory, automatic and central nervous system, somatomotor activity and behaviour pattern, tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.

(6) Body weight

Individual animals weights were recorded at the acquisition, grouping, and on the 1st, 3rd, 7th and 14th day after exposure.

(7) Necropsy and gross findings examination

On necropsy, all animals were anesthetized with dose of 1 ㎖/kg pentobarbital, and then terminated by exsanguination from the aorta.

Complete post-mortem, gross finding examinations were performed on all vital organs (subcutaneous, abdominal, thoracic and brain).

(8) Statistical analysis

Body weight and results of lung function test were analyzed by one way analysis of variance (ANOVA) for the three experimental groups with those for fresh-air control rats at $p < 0.05$ significance level. When the one-way ANOVA test suggested the significant differences at the level of $p<0.05$, Dunnett's test was employed to find the statistical meanings between control and exposure groups. The gross findings were expressed as frequency. Statistical parameters were expressed as mean and standard error (S.E).

(9) Compliance with the guidelines of animal ethics

This study was approved by the IACUC of Korea Conformity Laboratory (approval number : IA13-00221).

4. RESULTS

1) Environment for animal exposure chamber (Table 1 and Appendix 6)

The temperature, humidity, pressure, t_{95} and oxygen concentration in exposure day were recorded as 23.31±0.12 ℃, 41.60±0.64 %, -0.02±0.00 ㎜H_2O, 46.40±0.05 min and 21.25±0.01 % in chamber 1 (control group), and 23.01±0.12 ℃, 43.35±0.69 %, -0.07±0.00 ㎜H_2O, 46.30±0.06 min and 20.72±0.01 % in chamber 2 (low dose group). Chamber 3 (middle dose group) was recorded as 23.04±0.11 ℃, 46.55±0.44 %, -0.07±0.00 ㎜H_2O, 47.28±0.06 min and 20.41±0.01 %, and chamber 4 (high dose group) was recorded as 22.46±0.09 ℃, 57.36±0.59 %, -0.24±0.01 ㎜H_2O, 46.38±0.06 min and 20.46±0.01 %.

2) Distribution of MWCNT in exposure chamber
(Table 2, 3 and Appendix 7, 9)
The mass concentration of test substance was recorded as 0.17±0.00 ㎎/㎥, 0.52±0.01 ㎎/㎥, 0.83±0.01 ㎎/㎥ for low, middle and high dose respectively. The particle number of clean air entering the control chamber was 0.071±0.003 particles/㎤ in Channel 1(0.3 ㎛) and was 0.008±0.000 particles/㎤ in Channel 2(1.0 ㎛) of the particle sensor.

3) TEM analysis of test substance (Appendix 8)
The results of TEM analylsis, the shape was shown as fiber shape, and the main element was carbon through the analysis of energy dispersive X-ray spectrometer (EDX). Geometric mean (GM) and geometric standard deviation (GSD) of the cumulative median length (CML) were [redacted] ㎚ and [redacted], respectively.

4) Clinical signs (Table 4, 5 and Appendix 10, 11)
No toxic signs or mortality were observed relating to the test substance after MWCNT exposure during the observation period.

5) Body weight changes (Table 6, 7, Figure 4, 5 and Appendix 12, 13)
There was no significant difference in body weight change between control and exposure groups for male and female rats.

6) Gross findings examination (Table 8, 9 and Appendix 14, 15)
There were no observed specific clinical signs related to test substance in male and female rats.

5. DISCUSSION AND CONCLUSION

This study was performed to evaluate acute inhalation toxicity of MWCNT using specific pathogen free (SPF) - Fisher 344 (F344) rats with exposure concentration of 0, 0.17 (low-dose group), 0.52 (middle-dose group) and 0.83 (high-dose group) ㎎/㎥. The rats received a single event exposure to the test substance for 6 hours. There were 5 rats in each male and female group. Test method conformed to standard of OECD Guideline for Testing of Chemicals No. 403 'Acute Inhalation Toxicity' (Adopted 7th Sep., 2009).

Mortalities, clinical signs and body weight changes of experimental animals were evaluated during the observation period of 14 days. After termination of study, organ gross findings for the surviving animals were observed in necropsy. The results were as follows,

No toxic signs or mortality were observed relating to the test substance.
There was no significant difference of body weight changes between control and exposure groups.
At the end of study, all animals were subjected to necropsy, and no abnormal gross findings were observed in relation to the test substance.

In conclusion, LC_{50} value for the acute inhalation of MWCNT is considered to be > 0.83 ㎎/㎥ of mass concentration in this study.

6. REFERENCES

1) Guideline for the Testing of Chemical Hazards, National Institute of Environment Research (NIER) [Notice No. 2013-02 (revised 09th, Jan., 2013)]

2) OECD Guidelines for the Testing of Chemical No. 403 'Acute Inhalation Toxicity' (Adopted 7th Sep, 2009)

3) NIOSH, (1994). NIOSH manual of analytical methods, method No. 0500 'PARTICULATES NOT OTHERWISE REGULATED, TOTAL'. National Institute for Occupational Health, Cincinnati.

7. TABLES

Table 1. Environment of animal exposure chamber in acute inhalation toxicity study

ENVIRONMENT OF ANIMAL EXPOSURE CHAMBER					
STUDY ID : GT13-00173					Mean ± S.E
Group	Temperature (℃)	Humidity (%)	Pressure (mmH_2O)	t_{95}[a] (min)	Oxygen (%)
Control	23.31±0.12	41.61±0.64	-0.02±0.00	46.40±0.05	21.25±0.01
Low	23.01±0.12	43.35±0.69	-0.07±0.00	46.30±0.06	20.72±0.01
Medium	23.04±0.11	46.55±0.44	-0.07±0.00	47.28±0.06	20.41±0.01
High	22.46±0.09	57.36±0.59	-0.24±0.01	46.38±0.06	20.46±0.01

a : 3×(chamber volume/chamber airflow)

Table 2. Concentration of MWCNT in acute inhalation toxicity study

CONCENTRATION OF MWCNT		
STUDY ID : GT13-00173		Mean ± S.E
Group	Target Conc.	Mass (mg/m^3)
Control	0	0.00±0.00
Low	0.14~0.26 mg/m^3	0.17±0.00
Medium	0.35~0.65 mg/m^3	0.52±0.01
High	0.70~1.30 mg/m^3	0.83±0.01

Table 3. Distribution of particle in vehicle

DISTRIBUTION OF PARTICLE IN VEHICLE				
STUDY : GT13-00173				mean±S.E
	0.3 ㎛/min	1.0 ㎛/min	0.3 particle/cc	1.0 particle/cc
	200.70 ± 7.23 (361)	22.59 ± 0.88 (361)	0.071 ± 0.003 (361)	0.008 ± 0.000 (361)

Table 4. Clinical signs of male rats in acute inhalation toxicity study

CLINICAL SIGNS SUMMARY					
GT13-00173		MALE			
Day	SIGN	Group			
		Control	Low	Medium	High
0[a]	Normal	5/5	5/5	5/5	5/5
1	Normal	5/5	5/5	5/5	5/5
2	Normal	5/5	5/5	5/5	5/5
3	Normal	5/5	5/5	5/5	5/5
4	Normal	5/5	5/5	5/5	5/5
5	Normal	5/5	5/5	5/5	5/5
6	Normal	5/5	5/5	5/5	5/5
7	Normal	5/5	5/5	5/5	5/5
8	Normal	5/5	5/5	5/5	5/5
9	Normal	5/5	5/5	5/5	5/5
10	Normal	5/5	5/5	5/5	5/5
11	Normal	5/5	5/5	5/5	5/5
12	Normal	5/5	5/5	5/5	5/5
13	Normal	5/5	5/5	5/5	5/5
14	Normal	5/5	5/5	5/5	5/5

a : after exposure

Table 5. Clinical signs of female rats in acute inhalation toxicity study

CLINICAL SIGNS SUMMARY					
GT13-00173		FEMALE			
Day	SIGN	Group			
		Control	Low	Medium	High
0[a]	Normal	5/5	5/5	5/5	5/5
1	Normal	5/5	5/5	5/5	5/5
2	Normal	5/5	5/5	5/5	5/5
3	Normal	5/5	5/5	5/5	5/5
4	Normal	5/5	5/5	5/5	5/5
5	Normal	5/5	5/5	5/5	5/5
6	Normal	5/5	5/5	5/5	5/5
7	Normal	5/5	5/5	5/5	5/5
8	Normal	5/5	5/5	5/5	5/5
9	Normal	5/5	5/5	5/5	5/5
10	Normal	5/5	5/5	5/5	5/5
11	Normal	5/5	5/5	5/5	5/5
12	Normal	5/5	5/5	5/5	5/5
13	Normal	5/5	5/5	5/5	5/5
14	Normal	5/5	5/5	5/5	5/5

a : after exposure

Table 6. Body weights of male rats in acute inhalation toxicity study

SUMMARY OF BODY WEIGHTS				
STUDY : GT13-00173	UNIT : g			SEX : MALE
GROUP : (mean±S.E)	Control	Low	Medium	High
0 Day	148.12 ± 3.54 (5)	149.14 ± 3.42 (5)	149.07 ± 3.46 (5)	149.32 ± 3.63 (5)
1 Day	159.35 ± 2.96 (5)	159.49 ± 2.09 (5)	160.72 ± 4.00 (5)	158.30 ± 3.39 (5)
3 Day	169.33 ± 2.98 (5)	168.50 ± 1.94 (5)	169.71 ± 4.18 (5)	165.47 ± 3.43 (5)
7 Day	189.37 ± 2.80 (5)	189.37 ± 1.48 (5)	190.92 ± 3.61 (5)	183.51 ± 3.38 (5)
14 Day(Sacrifice)	217.22 ± 2.39 (5)	216.73 ± 1.03 (5)	221.79 ± 5.08 (5)	208.88 ± 2.51 (5)

( ) : animal number

Table 7. Body weights of female rats in acute inhalation toxicity study

SUMMARY OF BODY WEIGHTS				
STUDY : GT13-00173	UNIT : g			SEX : FEMALE
GROUP : (mean±S.E)	Control	Low	Medium	High
0 Day	125.81 ± 3.39 (5)	126.90 ± 2.93 (5)	127.44 ± 2.65 (5)	127.97 ± 2.55 (5)
1 Day	129.46 ± 3.28 (5)	131.17 ± 3.45 (5)	130.28 ± 2.59 (5)	132.25 ± 2.20 (5)
3 Day	134.03 ± 2.81 (5)	135.69 ± 3.37 (5)	135.12 ± 2.56 (5)	135.74 ± 1.68 (5)
7 Day	143.68 ± 3.03 (5)	146.30 ± 3.15 (5)	142.45 ± 2.96 (5)	144.40 ± 1.91 (5)
14 Day(Sacrifice)	152.43 ± 3.54 (5)	152.68 ± 3.10 (5)	151.41 ± 3.74 (5)	151.23 ± 1.52 (5)

( ) : animal number

Table 8. Gross findings of male rats in acute inhalation toxicity study

SUMMARY OF GROSS FINDINGS

STUDY : GT13-00173 SEX : MALE

ORGAN	OBSERVATION	GROUP			
		Control	Low	Medium	High
TESTIS (LEFT)	Normal	5/5	5/5	5/5	5/5
TESTIS (RIGHT)	Normal	5/5	5/5	5/5	5/5
KIDNEY (LEFT)	Normal	5/5	5/5	5/5	5/5
KIDNEY (RIGHT)	Normal	5/5	5/5	5/5	5/5
SPLEEN	Normal	5/5	5/5	5/5	5/5
LIVER	Normal	5/5	5/5	5/5	5/5
ADRENAL GLAND (LEFT)	Normal	5/5	5/5	5/5	5/5
ADRENAL GLAND (RIGHT)	Normal	5/5	5/5	5/5	5/5
HEART	Normal	5/5	5/5	5/5	5/5
THYMUS	Normal	5/5	5/5	5/5	5/5
LUNG (LEFT)	Normal	5/5	5/5	5/5	5/5
LUNG (RIGHT)	Normal	5/5	5/5	5/5	5/5
INTESTINE	Normal	5/5	5/5	5/5	5/5
CERVICAL LYMPHNODE	Normal	5/5	5/5	5/5	5/5
VENA CAVA CAUDALIS	Normal	5/5	5/5	5/5	5/5
BLADDER	Normal	5/5	5/5	5/5	5/5
BRAIN	Normal	5/5	5/5	5/5	5/5
OLFACTORY BULB	Normal	5/5	5/5	5/5	5/5

Table 9. Gross findings of female rats in acute inhalation toxicity study

SUMMARY OF GROSS FINDINGS

STUDY : GT13-00173 SEX : FEMALE

ORGAN	OBSERVATION	GROUP			
		Control	Low	Medium	High
OVARY (LEFT)	Normal	5/5	5/5	5/5	5/5
OVARY (RIGHT)	Normal	5/5	5/5	5/5	5/5
KIDNEY (LEFT)	Normal	5/5	5/5	5/5	5/5
KIDNEY (RIGHT)	Normal	5/5	5/5	5/5	5/5
SPLEEN	Normal	5/5	5/5	5/5	5/5
LIVER	Normal	5/5	5/5	5/5	5/5
ADRENAL GLAND (LEFT)	Normal	5/5	5/5	5/5	5/5
ADRENAL GLAND (RIGHT)	Normal	5/5	5/5	5/5	5/5
HEART	Normal	5/5	5/5	5/5	5/5
THYMUS	Normal	5/5	5/5	5/5	5/5
LUNG (LEFT)	Normal	5/5	5/5	5/5	5/5
LUNG (RIGHT)	Normal	5/5	5/5	5/5	5/5
INTESTINE	Normal	5/5	5/5	5/5	5/5
CERVICAL LYMPHNODE	Normal	5/5	5/5	5/5	5/5
VENA CAVA CAUDALIS	Normal	5/5	5/5	5/5	5/5
BLADDER	Normal	5/5	5/5	5/5	5/5
BRAIN	Normal	5/5	5/5	5/5	5/5
OLFACTORY BULB	Normal	5/5	5/5	5/5	5/5

8. FIGURE

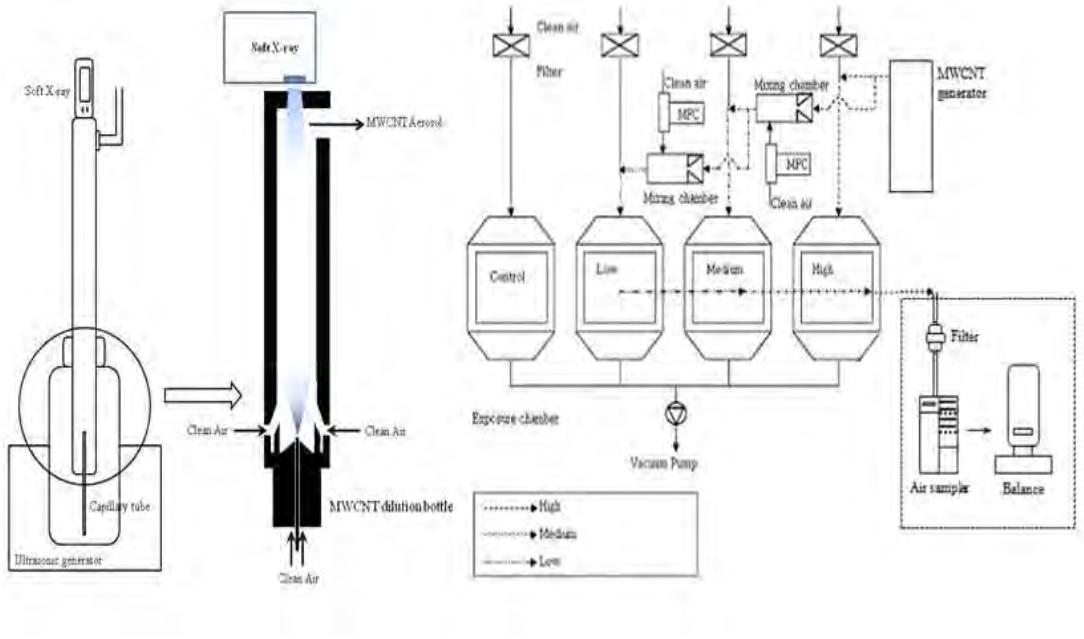


Figure 1. Schematic diagram of inhalation toxicity study

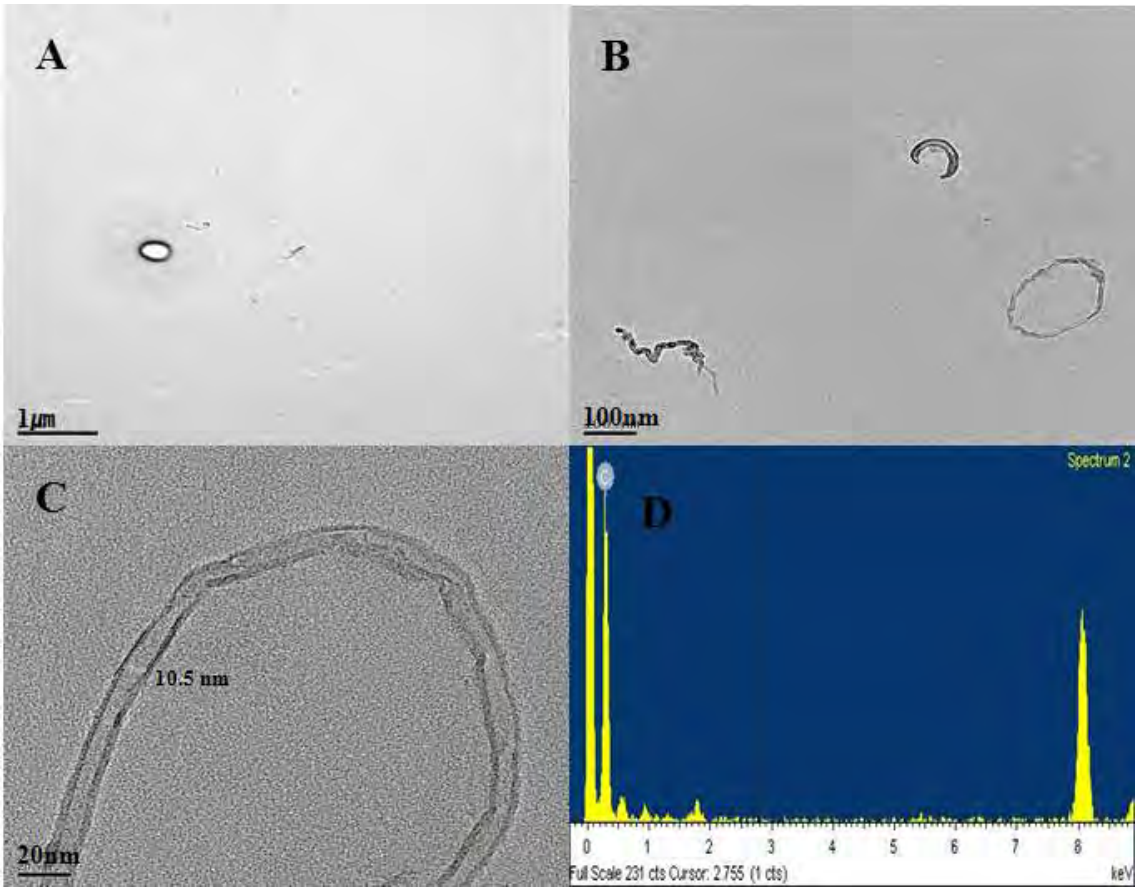


Figure 2. MWCNT by TEM

A~C : Scanning Transmission Electron Microscope (×100,000)

D : EDX spectometer

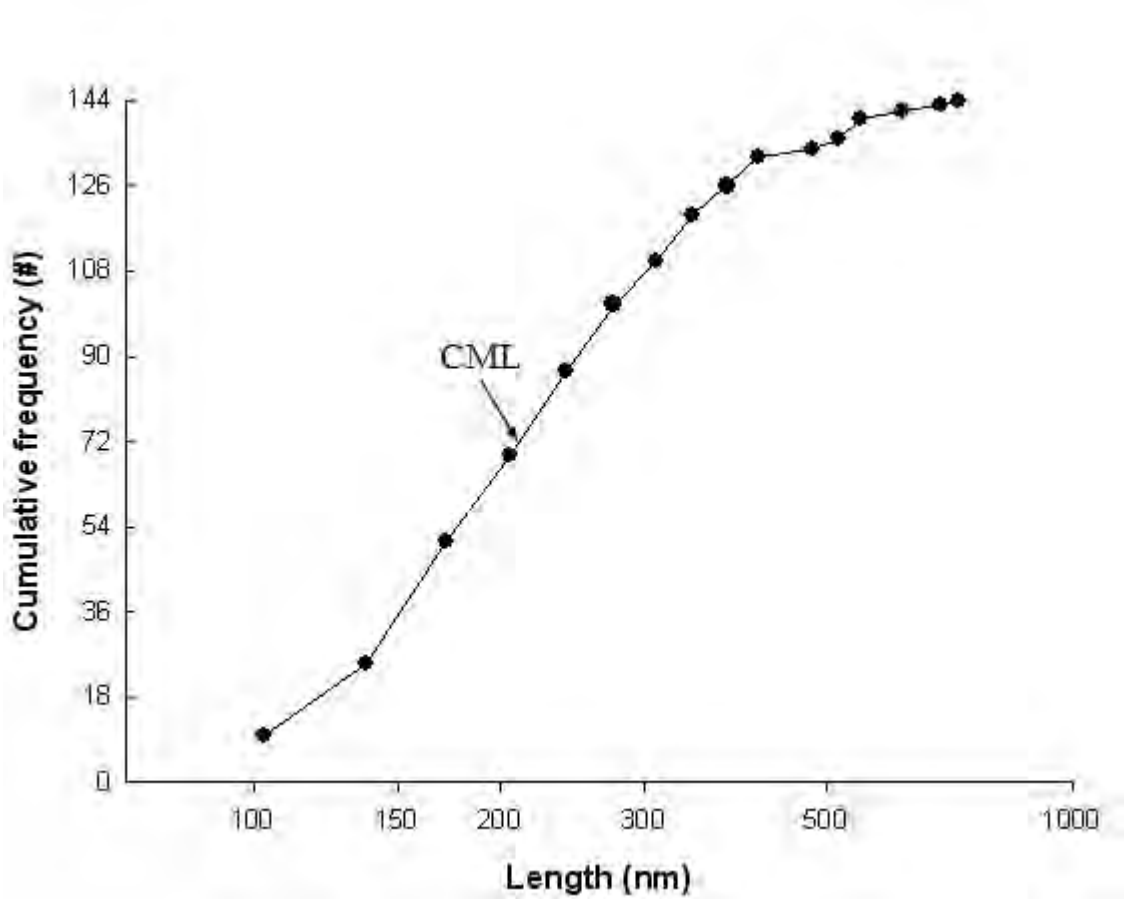


Figure 3. Cumulative mean length of MWCNT

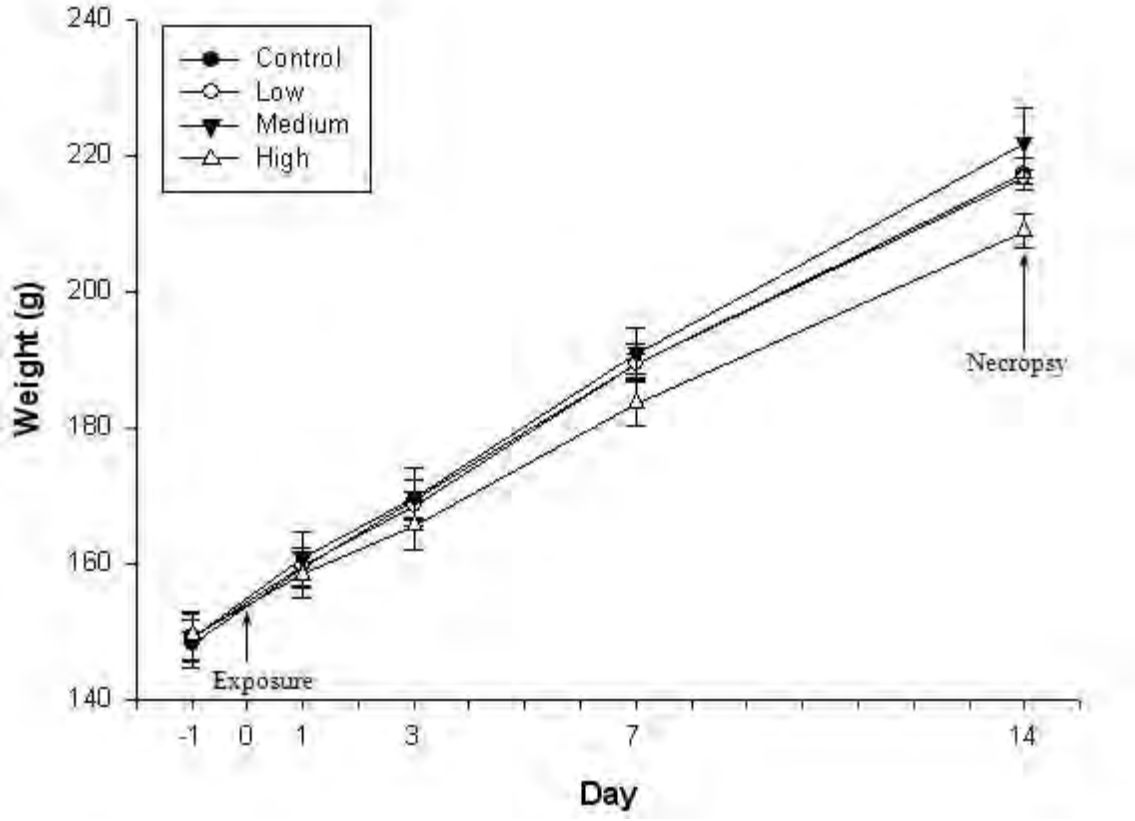


Figure 4. Body weight changes of male rats in acute inhalation toxicity study

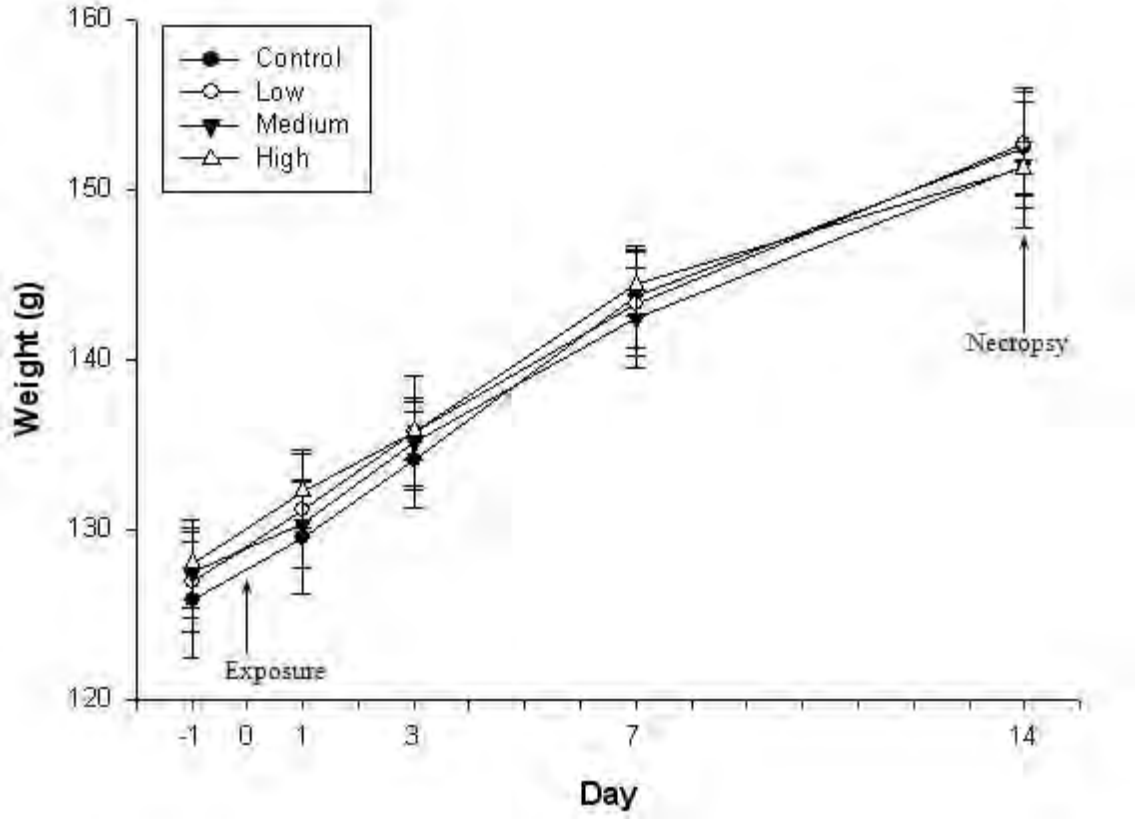
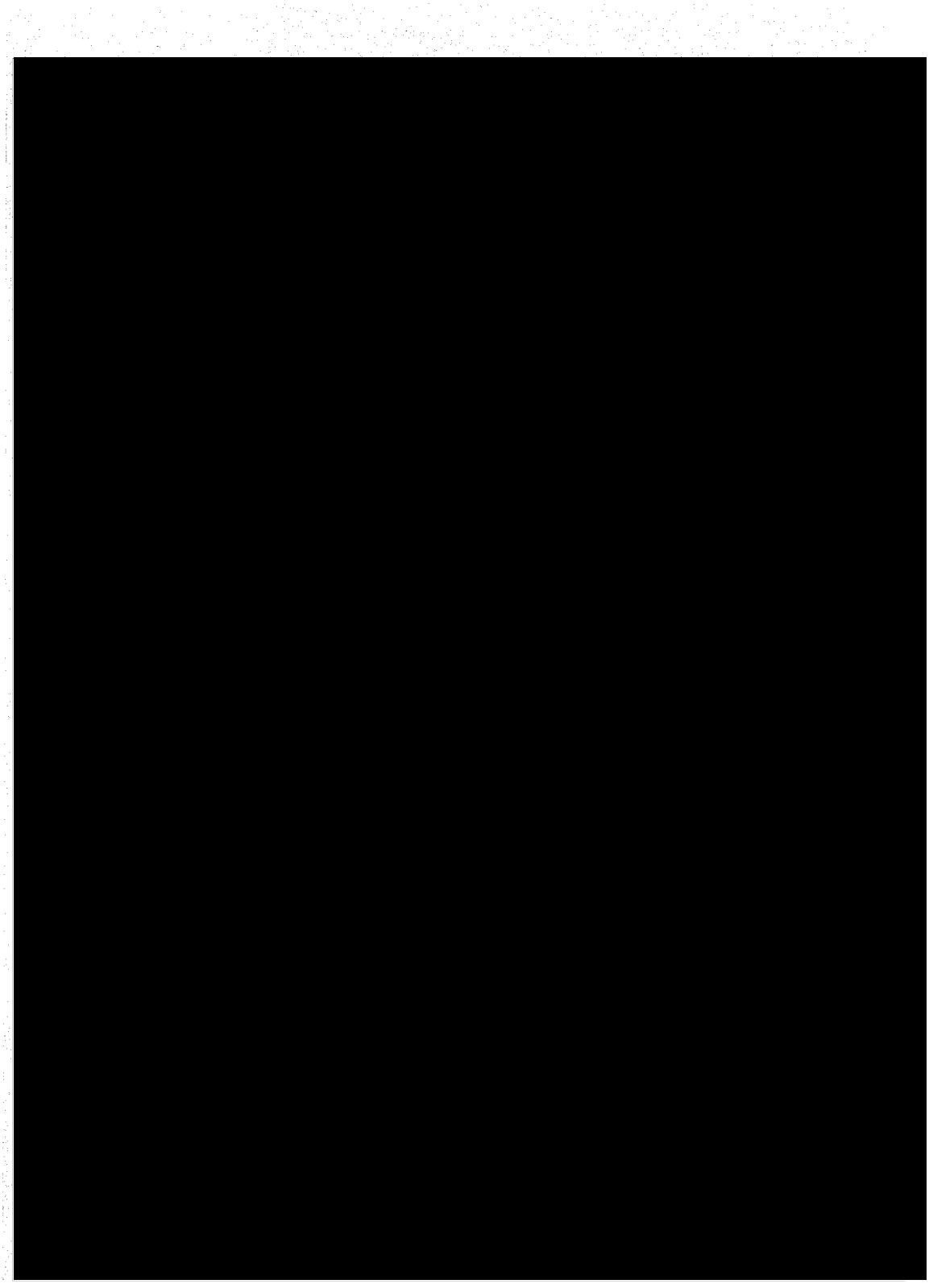


Figure 5. Body weight changes of female rats in acute inhalation toxicity study

9. APPENDICES

Appendix 1. Test substance data sheet



Appendix 2. Animals diagnostic report

GT13-00173

HEALTH MONITORING REPORT

April 30, 2018

Production facility	Inasa (SPF rats)																Inasa (SPF mice)									
Strain	Slc:SD			Slc:Wistar/ST			F344/NSlc			LEW/SsNSlc			SHR/Izm			WKY/Izm			Slc:ddY			Slc:ICR		
Sacrificed day	3 / 27			3 / 26			3 / 21			3 / 21			3 / 18			3 / 25			3 / 26			3 / 27		
Test	a	b	c. d	a	b	c. d	a	b	c. d	a	b	c. d	a	b	c. d	a	b	c. d	a	b	c. d	a	b	c. d
Age and number of tested animals	Re 30 6W 10	Re 20	Re 10	Re 30 6, 7W 10	Re 20	Re 10	Re 6W 5	Re 5	Re 5	Re 6W 5	Re 10	Re 5	Re 6W 6	Re 6	Re 6	Re 5W 6	Re 6	Pe 6	Re 6W 10	Re 40	Re 40		Re 20	Re 20	
Items																									
Pseudomonas aeruginosa	0			0			0			0			0			0			0				0		
Citrobacter rodentium																			0				0		
Salmonella spp.	0			0			0			0									0				0		
Pasteurella pneumotropica		0			0			0			0		0			0			0		0		0		
Bordetella bronchiseptica	0			0			0			0			0			0			0				0		
Streptococcus pneumoniae	0			0			0			0			0			0									
Corynebacterium kutscheri	0	0			0			0			0														
Clostridium piliforme	0			0	0		0	0		0	0		0	0		0	0		0	0			0	0	
CAR bacillus		0	0		0	0		0	0		0	0		0	0		0	0			0			0	
Helicobacter hepaticus																									
Helicobacter bilis			0			0			0			0			0			C							
Mycoplasma pulmonis	0		0	0		0			0			0			0			0							
Mouse hepatitis virus																									
Sialodacryoadenitis virus		0	0		0	0		0	0		0	0		0	0		0	0		0			0		
Sendai virus		0	0		0	0		0	0		0	0		0	0		0	0							
Hantavirus		0	0		0	0		0	0		0	0		0	0		0	0		0			0		
Pneumonia virus of mice																									
Lymphocytic choriomeningitis																									
Mouse adenovirus																									
Ectromelia virus			0			0			0			0			0			0							
Murine norovirus																									
Pinworm	0			0			0			0			0			0			0			0			
Intestinal protozoa	0			0			0			0			0			0			0			0			
Ectoparasits	0			0			0			0			0			0			0			0			
Dermatophytes	0			0			0			0			0			0			0			0			

(Number of Positive)

Remarks : a = Bacteriology and Parasitology
b = Serology (CF or Aggl.)
c = Serology (EIA or IFA)
d = RT-nested PCR method
Re = Retired breeders

Japan SLC, Inc

Shouhei Takagi, D.V. M.
Director, Department of
Laboratory Animal Medicine

CERTIFICATE OF STRAIN

We hereby certify the strain of the animals and their background as follows

Place of birth :	Japan SLC, Inc. Inasa Production Facility
Purchaser :	
Shipping date and flight number :	January 10 ,2013
Monitoring result :	As attached sheet

Details:

	Inbred Rat			
	GK/Slc	F344/NSlc	HWY/Slc	LEW/SsNSlc
Origin & History	Tohoku University School of Medicine ⇒ 1999, SLC (F?) 2012, (F?+31)	NIH ⇒ ? , Jms ⇒ 1980, SLC (F138) 2012. (F138+70)	Yagi Memorial Park, Japan ⇒ 1993, SLC	NIH ⇒ 1994, SLC (F94) 2012, (F94+36)

Japan SLC, Inc.

S Takagi

Shouhei Takagi, D.V.M.
Director, Department of
Laboratory Animal Medicine

Genetic profiles for biochemical and immunogenetic markers of inbred rat strains

– July, 2011 to December, 2011 –

Strain	Chromosome No.	1	2	3	3	5	8	13	14	19	19	19	19	20
	Locus	*Hbb*	*Amy1*	*Cat*	*Hao1*	*Pgd*	*Gdc1*	*Fh*	*Gc*	*Es1*	*Es2*	*Es3*	*Es4*	*RT1.A**
ACI/NSlc		b	b	a	a	b	a	b	a	b	a	a	b	*a*
BN/SsNSlc		a	b	a	b	b	b	a	a	a	c	d	b	*n*
DA/Slc		b	a	a	a	b	a	b	a	b	a	a	b	*a*
DIR/Eis		b	b	a	a	b	a	b	a	b	d	c	b	*l*
DIS/Eis		b	a	a	a	b	a	b	a	b	d	c	b	*l*
EHBR/Eis		b	a	a	a	b	a	b	b	a	d	c	b	*l*
F344/NSlc		a	a	a	a	b	a	b	a	c	d	c	b	*l*
GK/Slc		a	a	a	a	b	a	b	a	a	a	a	b	*l*
HWY/Slc		b	a	a	a	b	a	b	a	a	d	c	b	*k*
LEW/SsNSlc		b	a	a	a	b	a	b	a	a	a	a	b	*d*
WBN/KobSlc		b	a	a	a	b	a	a	a	a	d	d	b	*l*
WKAH/HkmSlc		a	a	a	a	b	a	b	a	a	a	a	b	*u*
		b	a	b	a	b	a	b	a	a	a	a	b	*k*

RT1.A* : tested by Devision of Genetics, ICLAS Monitoring Center

Norio Masui, Ph.D.
Manager, Quality Control Department
Biotechnical Center, Japan SLC., Inc.

Appendix 3. Certification of Environment for animal care room

Certification of Environment for animal breeding room

Study No.	GT13-00173
Title	Acute Inhalation Toxicity Study of MWCNT in Fisher 344 rats (Acclimation period)
SPF Room No.	Inhalation toxicity animal room
Period of animal Breeding	2013-04-25 ~ 2013-04-29

Breeding environment condition

Section	Range of SOP	Survey value	Remark
Temperature	22±3 ℃	22.0±1.1 ℃	
Humidity	50±20 %RH	48.7±6.7% RH	
Luminous intensity	150~300 Lux	293 Lux	
Noise	60 db less than	47.3 dB	
Ammonia	15 ppm less than	5 ppm less than	

It is authenticated that there is no change of environment which digresses from the above established value for more than 2 hours during the test period.

Facility management director Dong-Seok Beck (sign)

2013-07-03

Appendix 3. Certification of Environment for animal care room (continued)

Certification of Environment for animal breeding room

Study No.	GT13-00173
Title	Acute Inhalation Toxicity Study of MWCNT in Fisher 344 rats (Exposure period)
SPF Room No.	Inhalation toxicity room #1
Period of animal Breeding	2013-04-30

Breeding environment condition

Section	Range of SOP	Survey value	Remark
Temperature	22±3 ℃	22.4±1.8 ℃	
Humidity	50±20 %RH	45.9±7.8% RH	
Luminous intensity	150~300 Lux	276 Lux	
Noise	60 db less than	58.9 dB	
Ammonia	15 ppm less than	5 ppm less than	

It is authenticated that there is no change of environment which digresses from the above established value for more than 2 hours during the test period.

Facility management director Dong-Seok Beck (sign)

2013-07-03

Appendix 3. Certification of Environment for animal care room (continued)

Certification of Environment for animal breeding room

Study No.	GT13-00173
Title	Acute Inhalation Toxicity Study of MWCNT in Fisher 344 rats (Clinical sign observation period)
SPF Room No.	Inhalation toxicity animal room
Period of animal Breeding	2013-05-01 ~ 2013-05-14

Breeding environment condition

Section	Range of SOP	Survey value	Remark
Temperature	22±3 ℃	21.8±0.9 ℃	
Humidity	50±20 %RH	51.5±4.2% RH	
Luminous intensity	150~300 Lux	293 Lux	
Noise	60 db less than	47.3 dB	
Ammonia	15 ppm less than	5 ppm less than	

It is authenticated that there is no change of environment which digresses from the above established value for more than 2 hours during the test period.

Facility management director Dong-Seok Beck (sign)

2013-07-03

Appendix 4. Laboratory animal diet certification report

Laboratory Diet Certification Report

Teklad Certified Irradiated Global 18% Protein Rodent Diet

2918C

Lot Number 2918C-120212MA

Date of Manufacture 12/02/12

Report Date 12/18/12

The following data is a consolidation of results obtained from one or more independent testing laboratories. The actual laboratory results are available upon request.

Kurt Schafer

Quality Assurance Coordinator, Teklad Diets
Research Models and Services
Harlan Laboratories, Inc.

I have reviewed this document
2012.12.21 07:18:28 -06'00'

Proximate Analysis

Analysis	Result (%)
Protein	18.40
Fat	6.14
Fiber	3.32
Moisture	12.00
Ash	6.61
Calcium	0.88
Phosphorus	0.68

Feed Contaminant Screen

Analysis	Result	Units	Established Maximum Concentration
Heavy Metals			
Arsenic	0.17	ppm	1.00
Cadmium	< 0.10	ppm	0.50
Lead	< 0.20	ppm	1.50
Mercury	< 0.05	ppm	0.20
Selenium	0.24	ppm	0.50
Mycotoxin			
Aflatoxin B1, B2, G1, G2	< 5.00	ppb	5.00
Chlorinated Hydrocarbons			
Aldrin	< 0.01	ppm	0.03
Lindane	< 0.01	ppm	0.05
Chlordane	< 0.01	ppm	0.05
DDT & related substances	< 0.03	ppm	0.15
Dieldrin	< 0.02	ppm	0.03
Endrin	< 0.02	ppm	0.03
Heptachlor	< 0.01	ppm	0.03
Heptachlor Epoxide	< 0.01	ppm	0.03
Toxaphene	< 0.10	ppm	0.15
PCB's	< 0.10	ppm	0.15
a-BHC	< 0.01	ppm	0.05
b-BHC	< 0.01	ppm	0.05
d-BHC	< 0.01	ppm	0.05
Hexachlorobenzene	< 0.01	ppm	0.03
Mirex	< 0.01	ppm	0.02
Methoxychlor	< 0.05	ppm	0.50
Organophosphates			
Thimet	< 0.15	ppm	0.50
Diazinon	< 0.14	ppm	0.50
Disulfaton	< 0.15	ppm	0.50
Methyl Parathion	< 0.14	ppm	0.50
Malathion	< 0.14	ppm	0.50
Parathion	< 0.12	ppm	0.50
Thiodan	< 0.02	ppm	0.50
Ethion	< 0.14	ppm	0.50
Trithion	< 0.15	ppm	0.50

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Appendix 4. Laboratory animal diet certification report (continued)

Laboratory Diet Certification Report

Teklad Certified Irradiated Global 18% Protein Rodent Diet

2918C

Lot Number 2918C-030413MA

Date of Manufacture 03/04/13

Report Date 03/19/13

The following data is a consolidation of results obtained from one or more independent testing laboratories. The actual laboratory results are available upon request.

Kurt Schafer

Quality Assurance Coordinator, Teklad Diets
Research Models and Services
Harlan Laboratories, Inc.

I have reviewed this document
2013.03.20 09:52:32 -05'00'

Proximate Analysis

Analysis	Result (%)
Protein	18.20
Fat	8.17
Fiber	3.82
Moisture	10.60
Ash	5.88
Calcium	1.01
Phosphorus	0.77

Feed Contaminant Screen

Analysis	Result	Units	Established Maximum Concentration
Heavy Metals			
Arsenic	0.12	ppm	1.00
Cadmium	< 0.10	ppm	0.50
Lead	< 0.20	ppm	1.50
Mercury	< 0.05	ppm	0.20
Selenium	0.34	ppm	0.50
Mycotoxin			
Aflatoxin B1, B2, G1, G2	< 5.00	ppb	5.00
Chlorinated Hydrocarbons			
Aldrin	< 0.01	ppm	0.03
Lindane	< 0.01	ppm	0.05
Chlordane	< 0.01	ppm	0.05
DDT & related substances	< 0.03	ppm	0.15
Dieldrin	< 0.02	ppm	0.03
Endrin	< 0.02	ppm	0.03
Heptachlor	< 0.01	ppm	0.03
Heptachlor Epoxide	< 0.01	ppm	0.03
Toxaphene	< 0.10	ppm	0.15
PCB's	< 0.10	ppm	0.15
a-BHC	< 0.01	ppm	0.05
b-BHC	< 0.01	ppm	0.05
d-BHC	< 0.01	ppm	0.05
Hexachlorobenzene	< 0.01	ppm	0.03
Mirex	< 0.01	ppm	0.02
Methoxychlor	< 0.05	ppm	0.50
Organophosphates			
Thimet	< 0.15	ppm	0.50
Diazinon	< 0.14	ppm	0.50
Disulfaton	< 0.15	ppm	0.50
Methyl Parathion	< 0.14	ppm	0.50
Malathion	< 0.14	ppm	0.50
Parathion	< 0.12	ppm	0.50
Thiodan	< 0.02	ppm	0.50
Ethion	< 0.14	ppm	0.50
Trithion	< 0.15	ppm	0.50

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Appendix 5. Certification of water analysis report

TEST REPORT

Reissuance (R1)
Date : 2013.4.11

1. No : PC13-00284
2. Client
   - Name : Korea Conformity Laboratories(Incheon)
   - Address : #7-44, Songdo-dong, Yeonsu-gu, Incheon, Korea
   - Date of Receipt : Mar. 14, 2013
   - Date of Issued : Apr. 17, 2013
3. Use of Report : Submission
4. Test Sample : Drinking Water (Animal room)
5. Method :
   (1) Notification No.2012-143 of the Ministry of Environment.

Affirmation	Tested By Name : Hyoung jun Seok Seok.	Technical Manager Name : Sang Cheul Lee S.C. Lee

Our report apply only to the standards or procedures identified and to the sample(s) tested unless otherwise specified.
The test results are not indicative of representative of the qualities of the qualities of the lot from which the sample was taken or of apparently identical or similar products.

Korea Conformity Laboratories President Song Jae Bin Jae Bin Song

Address : 704-932 277-5, Jukjeon-Dong, Dalseo-Gu, Daegu,704-932,Korea 82-53-557-6681
Result Inquiry : Environmental Testing Center 82-2-2102-2598

Appendix 5. Certification of water analysis report (continued)

TEST REPORT

No : PC13-00284

6. Test Results

1) Drinking Water (Animal room)

Test Item(s)	Unit	Limitation(s)	LOQ	Test method used	Test Result(s)
Total colony counts	CFU/mL	Less than 100	0	(1)	0
Total coliforms	-/(100mL)	Not detected	-	(1)	Not detected
E-Coli	-/(100mL)	Not detected	-	(1)	Not detected
Lead	mg/L	Less than 0.01	0.005	(1)	Not detected
Arsenic	mg/L	Less than 0.01	0.005	(1)	Not detected
Selenium	mg/L	Less than 0.01	0.005	(1)	Not detected
Cadmium	mg/L	Less than 0.005	0.002	(1)	Not detected
Bolon	mg/L	Less than 1.0	0.01	(1)	Not detected
Copper	mg/L	Less than 1.0	0.008	(1)	Not detected
Zinc	mg/L	Less than 3.0	0.002	(1)	0.003
Iron	mg/L	Less than 0.3	0.05	(1)	Not detected
Manganese	mg/L	Less than 0.3	0.005	(1)	Not detected
Aluminium	mg/L	Less than 0.2	0.02	(1)	Not detected
Mercury	mg/L	Less than 0.001	0.001	(1)	Not detected
Fluoride	mg/L	Less than 1.5	0.15	(1)	Not detected
Nitrate nitrogen	mg/L	Less than 10	0.1	(1)	0.2
Chloride	mg/L	Less than 250	0.4	(1)	0.6
Sulfate	mg/L	Less than 200	2	(1)	Not detected
Diazinon	mg/L	Less than 0.02	0.0005	(1)	Not detected
Parathion	mg/L	Less than 0.06	0.0005	(1)	Not detected
Fenitrotion	mg/L	Less than 0.04	0.0005	(1)	Not detected
Dichloromethane	mg/L	Less than 0.02	0.002	(1)	Not detected
1,1,1-Trichloroethane	mg/L	Less than 0.1	0.001	(1)	Not detected
Benzene	mg/L	Less than 0.01	0.001	(1)	Not detected
Toluene	mg/L	Less than 0.7	0.001	(1)	Not detected
Ethylbenzene	mg/L	Less than 0.3	0.001	(1)	Not detected
Xylene	mg/L	Less than 0.5	0.001	(1)	Not detected
1,1-Dichloroethylene	mg/L	Less than 0.03	0.001	(1)	Not detected
Tetrachlorocarbon	mg/L	Less than 0.002	0.001	(1)	Not detected
Tetrachloroethylene	mg/L	Less than 0.01	0.001	(1)	Not detected

Appendix 5. Certification of water analysis report (continued)

TEST REPORT

No. PC13-00284

6. Test Results

1) Drinking Water (Animal room)

Test Item(s)	Unit	Limitation(s)	LOQ	Test method used	Test Result(s)
Trichloroethylene	mg/L	Less than 0.03	0.001	(1)	Not detected
1,2-Dibromo-3-Chloropropane	mg/L	Less than 0.003	0.001	(1)	Not detected
Carbaryl	mg/L	Less than 0.07	0.005	(1)	Not detected
Chromium	mg/L	Less than 0.05	0.03	(1)	Not detected
Ammonium Nitrogen	mg/L	Less than 0.5	0.01	(1)	Not detected
Phenol	mg/L	Less than 0.005	0.005	(1)	Not detected
Alkyl Benzene Sulfate	mg/L	Less than 0.5	0.1	(1)	Not detected
Cyanide	mg/L	Less than 0.01	0.01	(1)	Not detected
pH	-	5.8 - 8.5	-	(1)	6.2
Turbidity	NTU	Less than 1	0.02	(1)	0.11
Color	degree	Less than 5	1	(1)	Not detected
Taste	-	Free	-	(1)	Pass
Odor	-	Free	-	(1)	Pass
Hardness	mg/L	Less than 300	1	(1)	Not detected
Consumption of $KMnO_4$	mg/L	Less than 10	0.3	(1)	0.6
Total solids	mg/L	Less than 500	2	(1)	Not detected

—— End of Report ——

Page 3 of 3

QP-20-01-08(2)

Appendix 6. Half hourly condition of exposure chamber in acute inhalation toxicity study

HALF HOURLY CONDITION OF EXPOSURE CHAMBER

GT13-00173

DAY : 2013/04/30

No	TIME	Control						Low					
		Temp	Humi	Pres	Flow	Oxy	t95[a)]	Temp	Humi	Pres	Flow	Oxy	t95
1	09:30	22.20	47.50	-0.06	45.50	21.15	46.15	21.90	49.90	-0.10	45.70	20.61	45.95
2	10:00	22.70	45.10	-0.03	45.40	21.20	46.26	22.40	46.90	-0.07	45.60	20.66	46.05
3	10:30	23.00	40.50	-0.03	45.40	21.22	46.26	22.70	43.50	-0.07	45.60	20.68	46.05
4	11:00	23.20	40.30	-0.03	45.50	21.25	46.15	22.90	41.90	-0.07	45.60	20.70	46.05
5	11:30	23.40	42.00	-0.02	45.40	21.25	46.26	23.00	43.40	-0.07	45.50	20.72	46.15
6	12:00	23.50	39.70	-0.01	45.20	21.26	46.46	23.20	41.40	-0.07	45.40	20.74	46.26
7	12:30	23.50	39.50	-0.01	45.10	21.28	46.56	23.20	41.50	-0.07	45.20	20.74	46.46
8	13:00	23.50	39.90	-0.01	45.20	21.27	46.46	23.20	41.60	-0.07	45.30	20.74	46.36
9	13:30	23.60	41.10	-0.01	45.10	21.27	46.56	23.30	42.60	-0.07	45.20	20.74	46.46
10	14:00	23.60	41.50	-0.01	45.10	21.28	46.56	23.30	42.90	-0.07	45.10	20.74	46.56
11	14:30	23.70	41.80	-0.01	45.10	21.28	46.56	23.40	43.20	-0.07	45.10	20.74	46.56
12	15:00	23.70	42.00	-0.01	45.00	21.26	46.67	23.40	43.90	-0.07	45.00	20.74	46.67
13	15:30	23.40	40.00	-0.01	45.40	21.29	46.26	23.20	40.90	-0.03	45.30	20.76	46.36

No	TIME	Medium						High					
		Temp	Humi	Pres	Flow	Oxy	t95	Temp	Humi	Pres	Flow	Oxy	t95
1	09:30	22.00	48.60	-0.10	44.70	20.34	46.98	21.60	54.30	-0.29	45.70	20.41	45.95
2	10:00	22.50	49.20	-0.07	44.60	20.37	47.09	22.00	58.20	-0.25	45.50	20.42	46.15
3	10:30	22.80	46.30	-0.07	44.60	20.38	47.09	22.20	56.90	-0.26	45.40	20.44	46.26
4	11:00	23.00	46.30	-0.07	44.60	20.40	47.09	22.40	56.50	-0.26	45.40	20.44	46.26
5	11:30	23.10	47.90	-0.07	44.50	20.42	47.19	22.50	58.80	-0.24	45.40	20.44	46.26
6	12:00	23.20	45.60	-0.07	44.30	20.42	47.40	22.60	58.00	-0.23	45.20	20.46	46.46
7	12:30	23.20	45.00	-0.07	44.20	20.42	47.51	22.60	57.00	-0.23	45.00	20.48	46.67
8	13:00	23.20	45.20	-0.07	44.40	20.42	47.30	22.60	56.50	-0.23	45.30	20.48	46.36
9	13:30	23.30	46.60	-0.07	44.30	20.42	47.40	22.70	57.60	-0.23	45.10	20.48	46.56
10	14:00	23.30	46.70	-0.07	44.30	20.42	47.40	22.70	59.50	-0.23	45.10	20.46	46.56
11	14:30	23.30	47.20	-0.07	44.20	20.42	47.51	22.70	59.40	-0.23	45.10	20.48	46.56
12	15:00	23.40	47.30	-0.07	44.10	20.42	47.62	22.70	60.40	-0.23	45.00	20.48	46.67
13	15:30	23.20	43.30	-0.04	44.60	20.44	47.09	22.70	52.60	-0.24	45.40	20.49	46.26

a : 3×(chamber volume/chamber airflow)

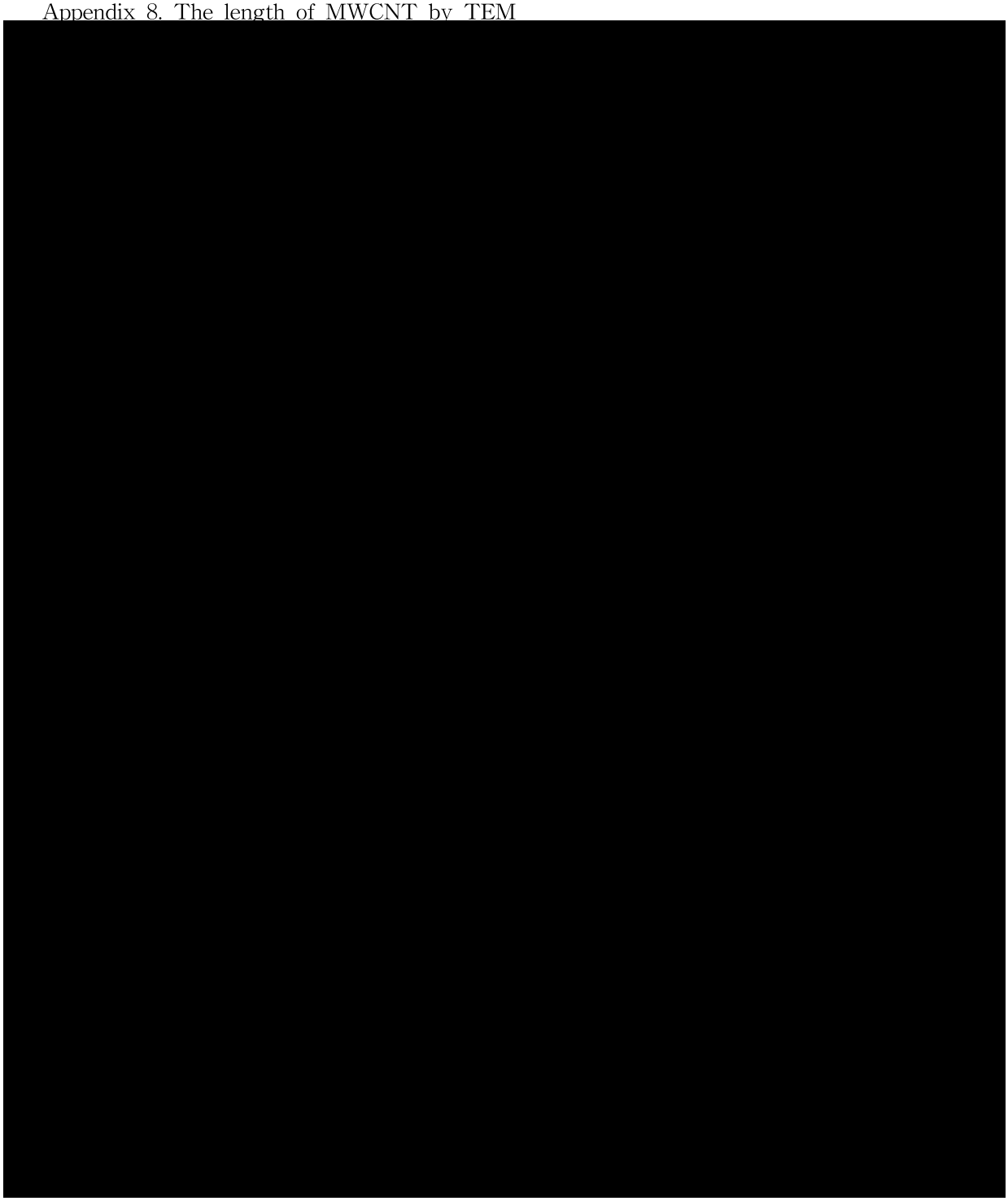
Appendix 7. Concentration of MWCNT in acute inhalation toxicity study

CONCENTRATION OF MWCNT

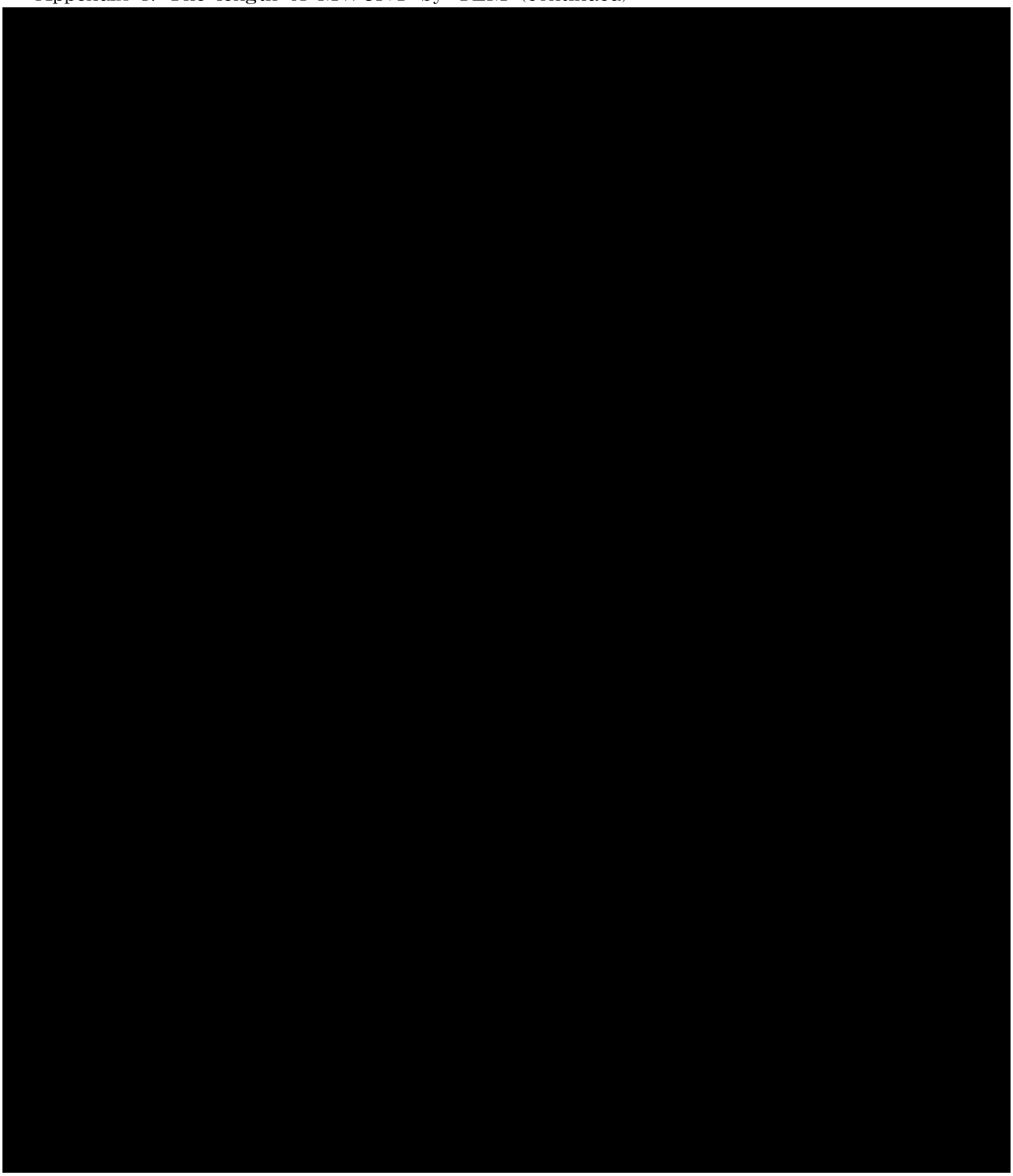
STUDY ID : GT13-00173 DAY : 2013/04/30

Group	Times	Mass (mg/m^3)
Low	1	0.173
	2	0.172
Medium	1	0.525
	2	0.508
High	1	0.819
	2	0.831

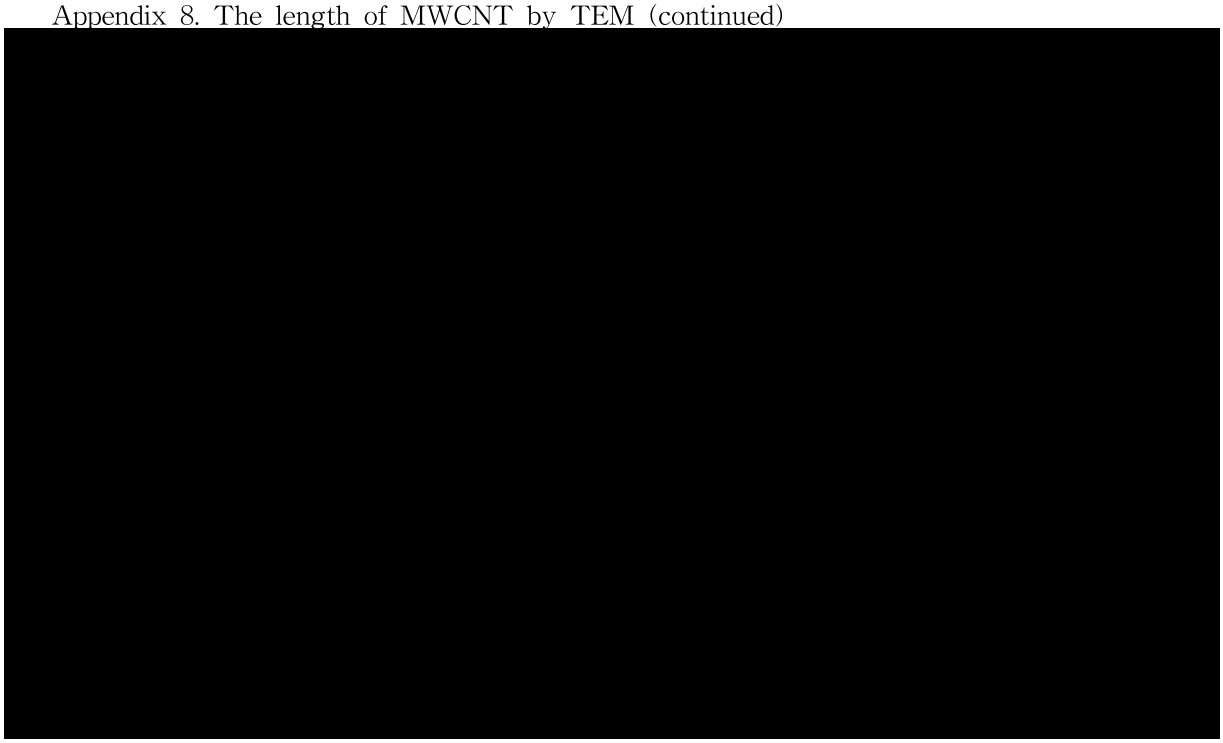
Appendix 8. The length of MWCNT by TEM



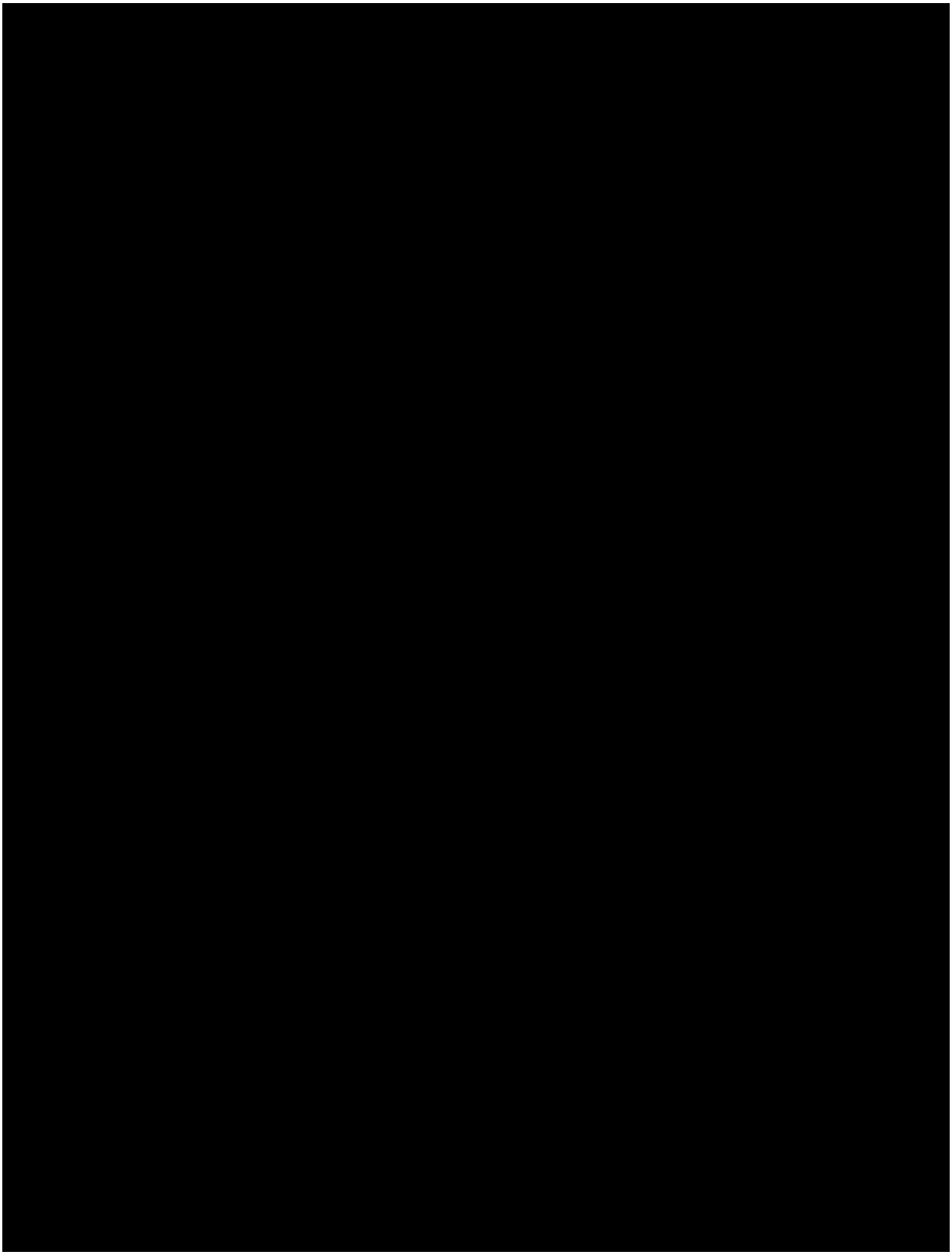
Appendix 8. The length of MWCNT by TEM (continued)



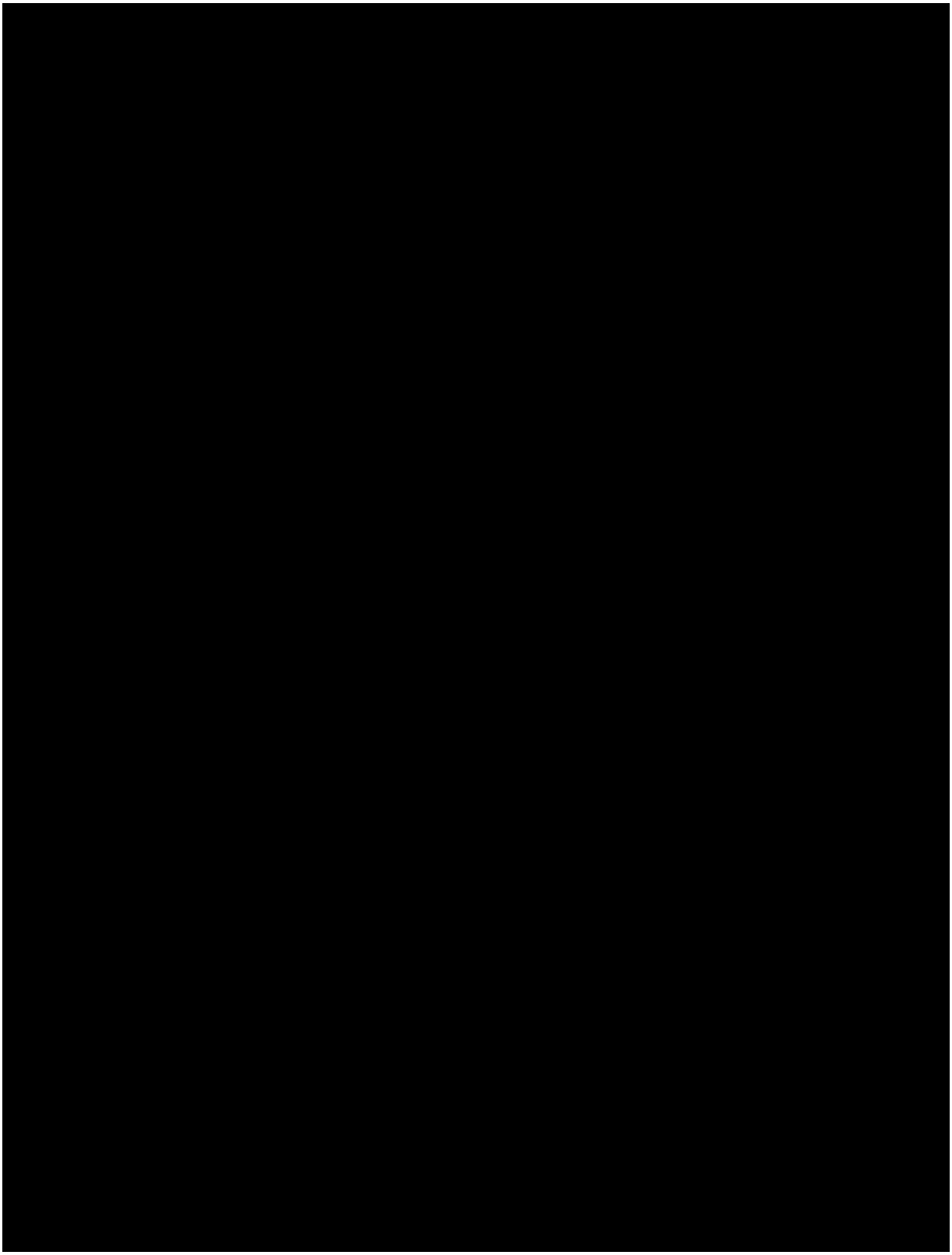
Appendix 8. The length of MWCNT by TEM (continued)



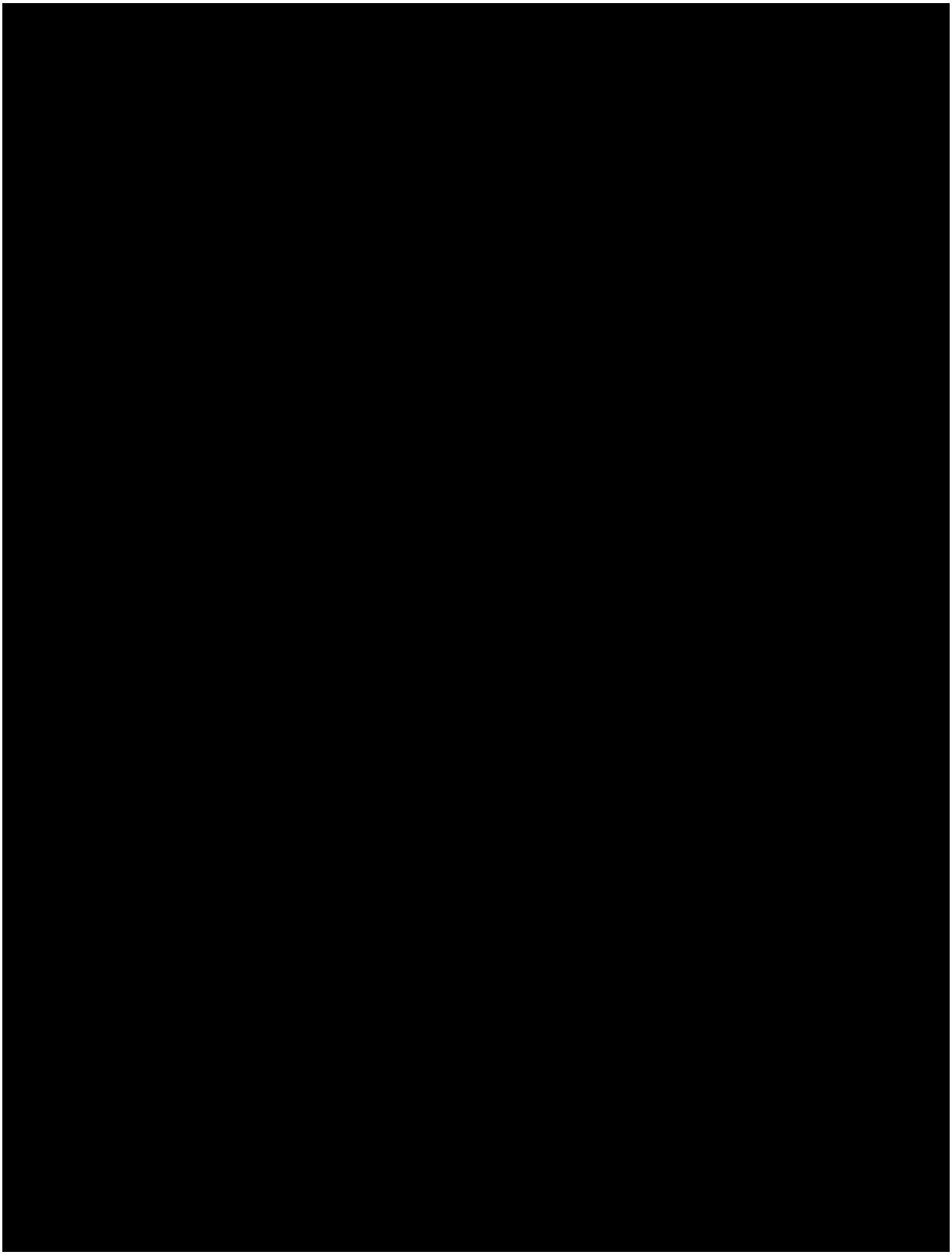
Appendix 9. Daily distribution of particle in vehicle



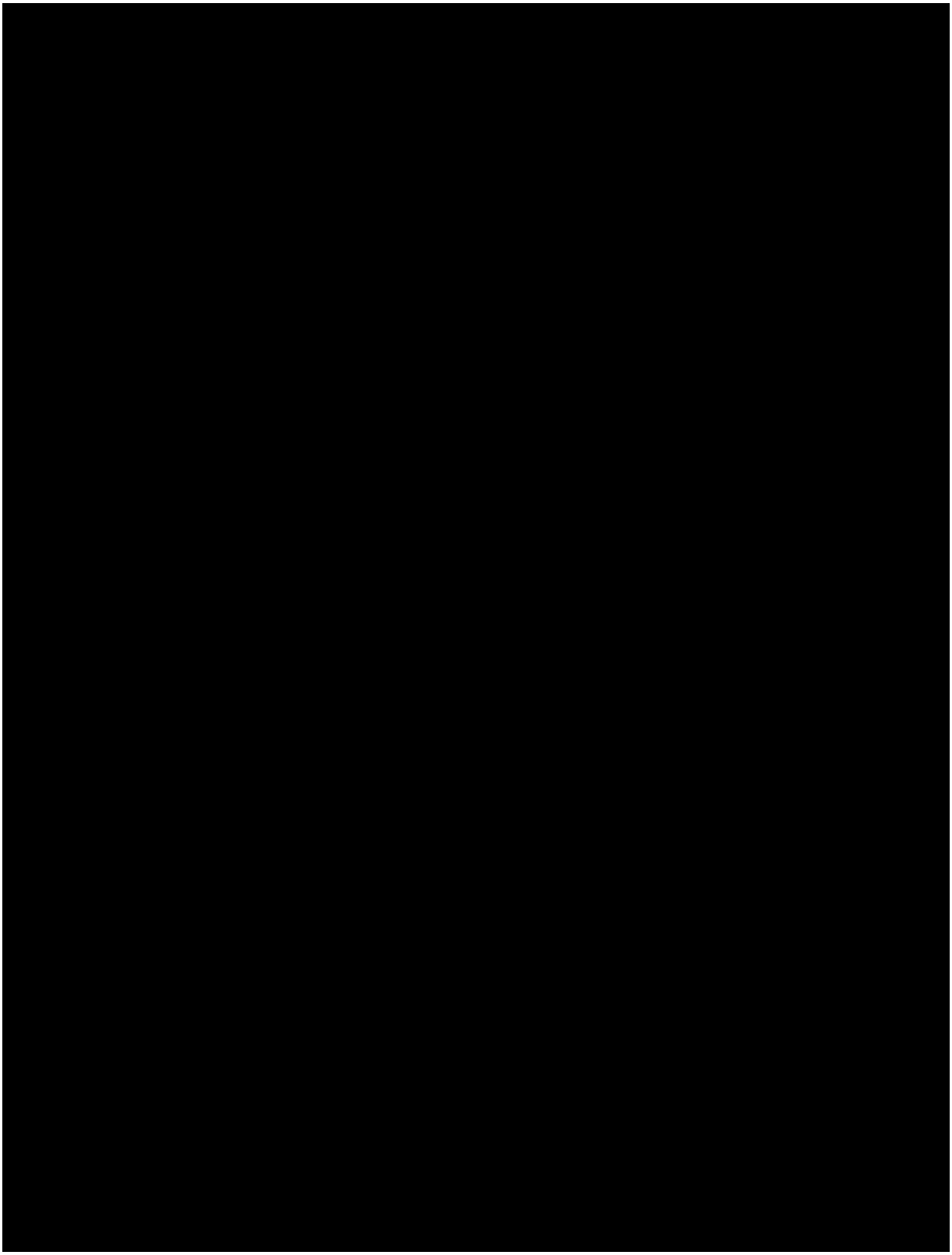
Appendix 9. Daily distribution of particle in vehicle (continued)



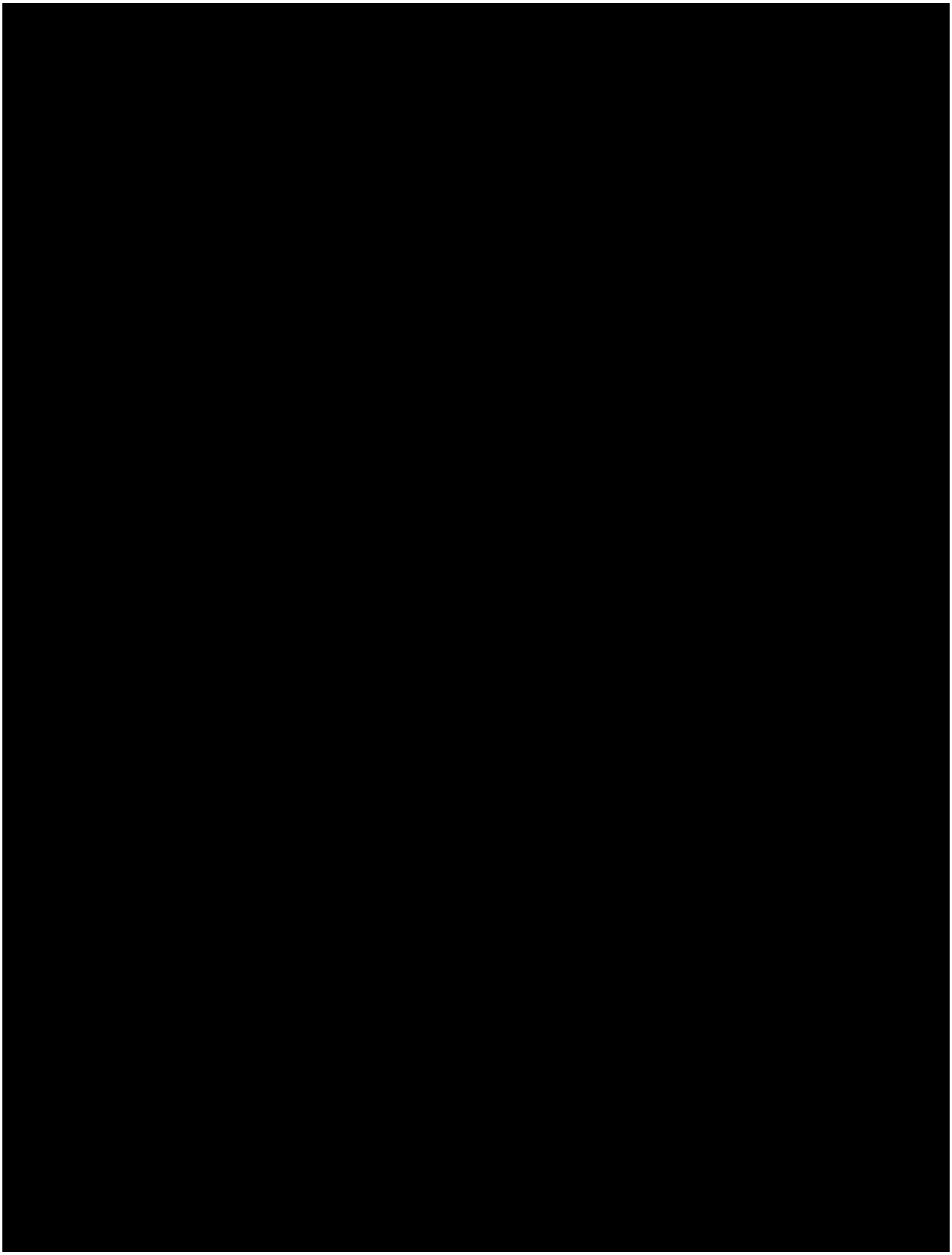
Appendix 9. Daily distribution of particle in vehicle (continued)



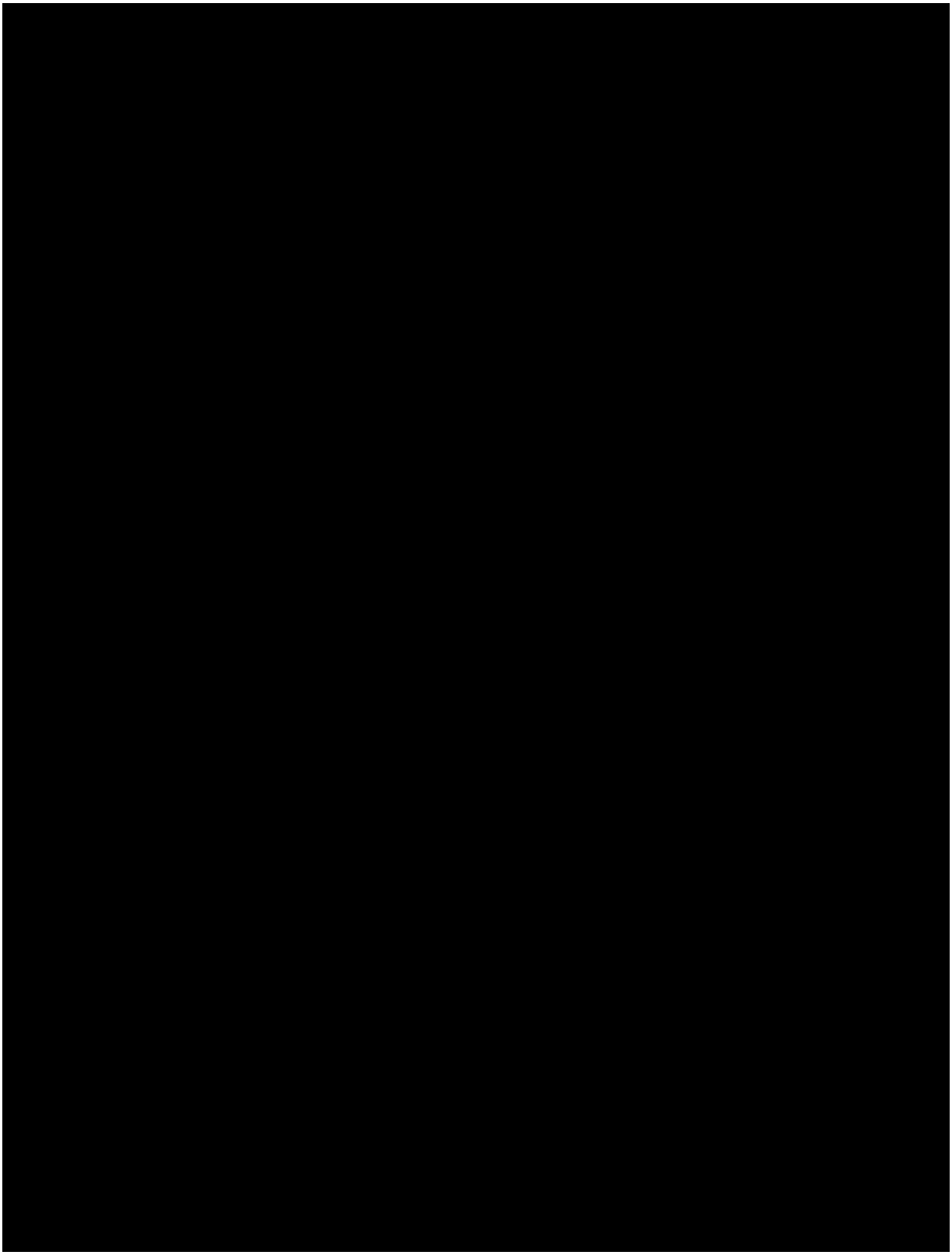
Appendix 9. Daily distribution of particle in vehicle (continued)



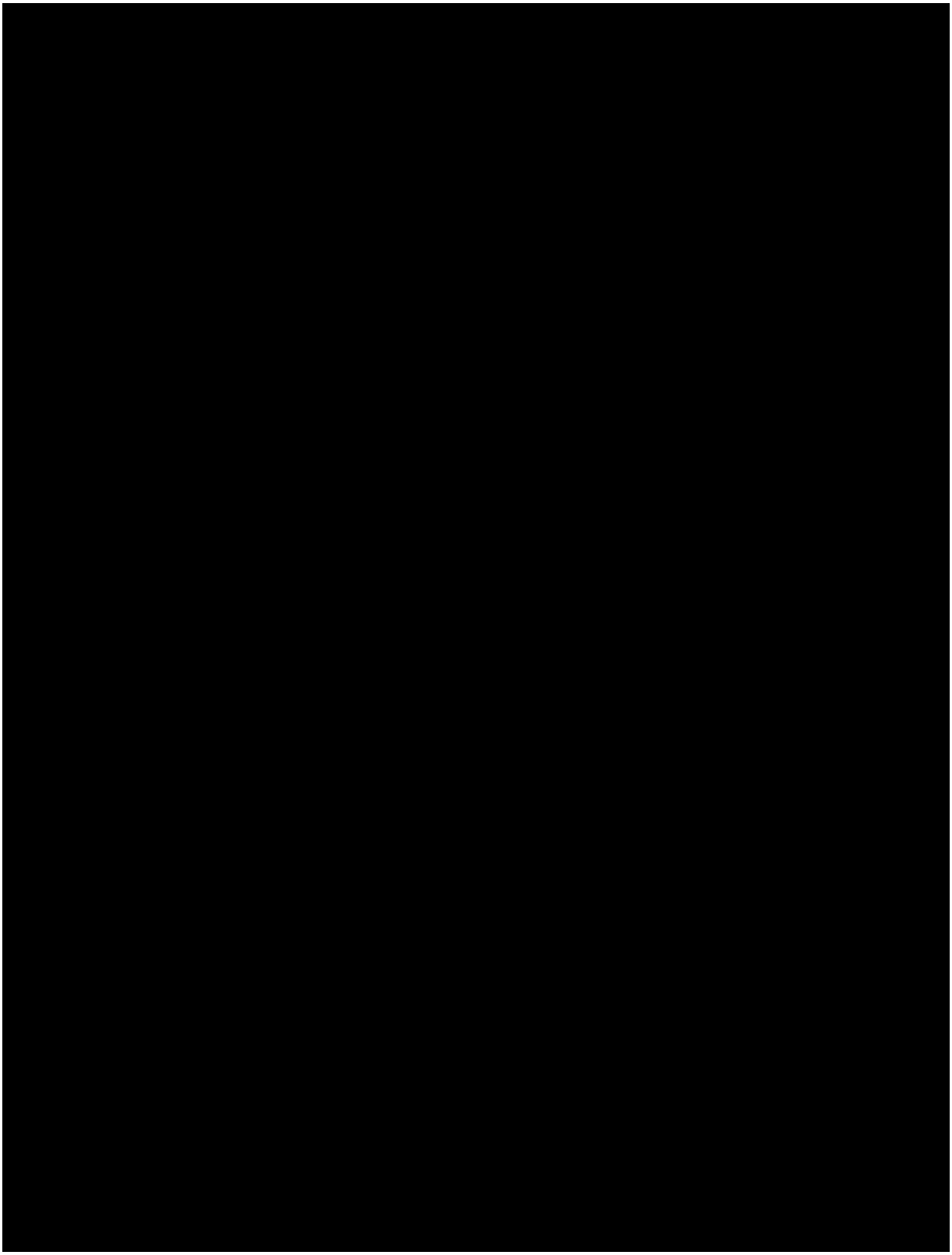
Appendix 9. Daily distribution of particle in vehicle (continued)



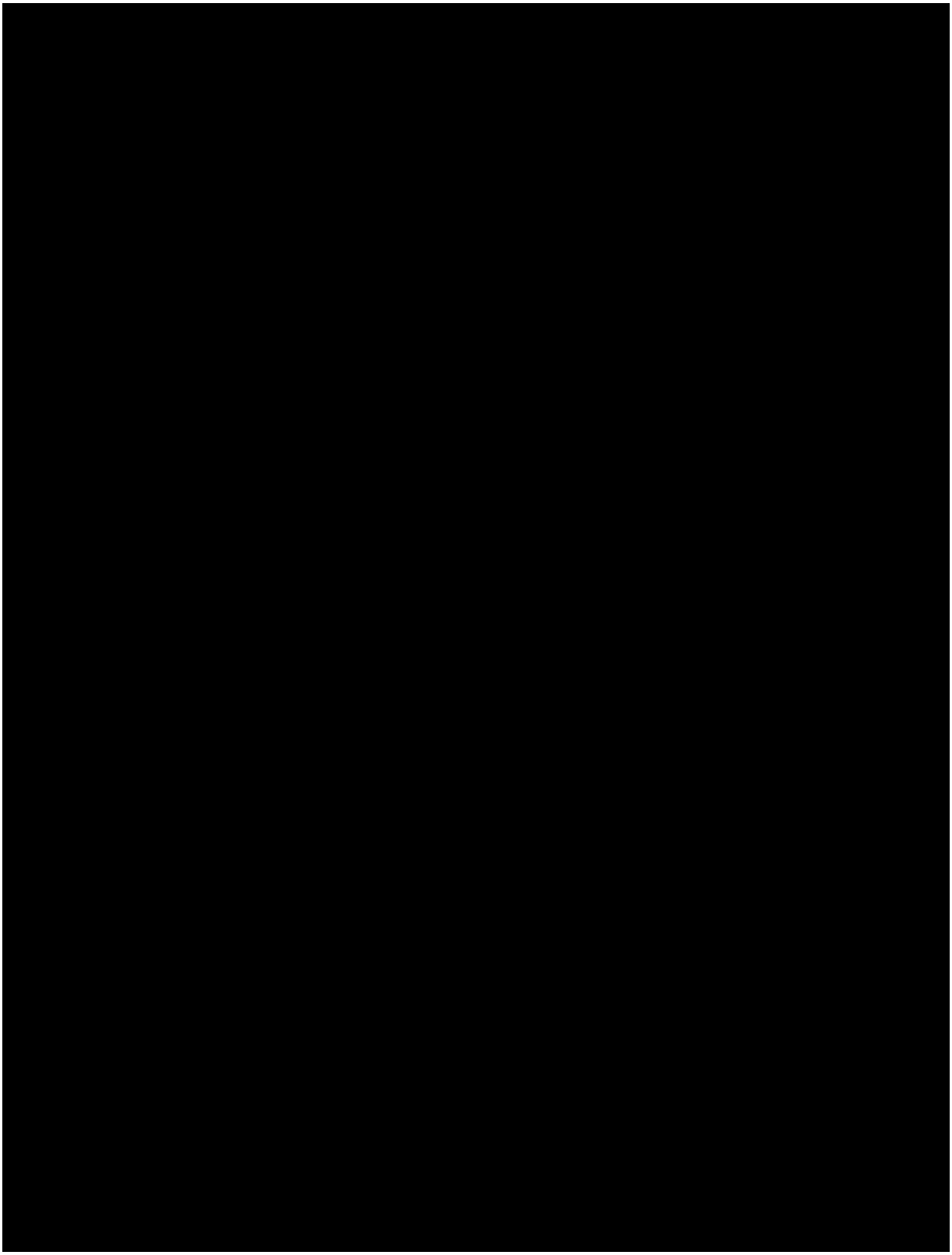
Appendix 9. Daily distribution of particle in vehicle (continued)



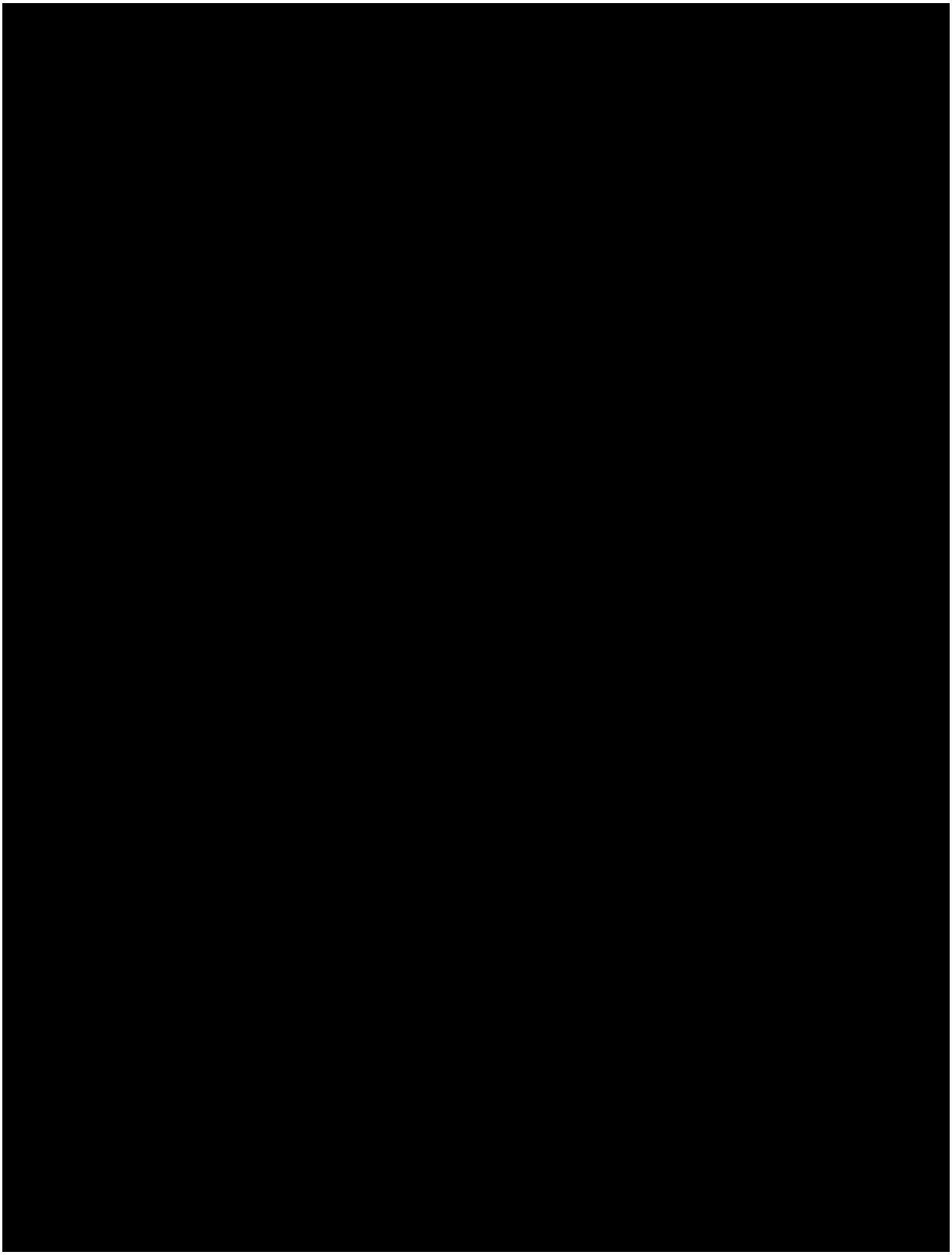
Appendix 9. Daily distribution of particle in vehicle (continued)



Appendix 9. Daily distribution of particle in vehicle (continued)



Appendix 9. Daily distribution of particle in vehicle (continued)



Appendix 10. Individual clinical signs in male rats in acute inhalation toxicity study

CLINICAL SIGNS INDIVIDUAL DATA		
GT13-00173		SEX : MALE
Animal No.	OBSERVATIONS	TIME OCCURED
1	Normal	0[a] - 14 Day
	Terminal sacrifice	14 Day
2	Normal	0 - 14 Day
	Terminal sacrifice	14 Day
3	Normal	0 - 14 Day
	Terminal sacrifice	14 Day
4	Normal	0 - 14 Day
	Terminal sacrifice	14 Day
5	Normal	0 - 14 Day
	Terminal sacrifice	14 Day
6	Normal	0 - 14 Day
	Terminal sacrifice	14 Day
7	Normal	0 - 14 Day
	Terminal sacrifice	14 Day
8	Normal	0 - 14 Day
	Terminal sacrifice	14 Day
9	Normal	0 - 14 Day
	Terminal sacrifice	14 Day
10	Normal	0 - 14 Day
	Terminal sacrifice	14 Day
11	Normal	0 - 14 Day
	Terminal sacrifice	14 Day
12	Normal	0 - 14 Day
	Terminal sacrifice	14 Day
13	Normal	0 - 14 Day
	Terminal sacrifice	14 Day
14	Normal	0 - 14 Day
	Terminal sacrifice	14 Day
15	Normal	0 - 14 Day
	Terminal sacrifice	14 Day
16	Normal	0 - 14 Day
	Terminal sacrifice	14 Day
17	Normal	0 - 14 Day
	Terminal sacrifice	14 Day
18	Normal	0 - 14 Day
	Terminal sacrifice	14 Day
19	Normal	0 - 14 Day
	Terminal sacrifice	14 Day
20	Normal	0 - 14 Day
	Terminal sacrifice	14 Day

a : after exposure

Appendix 11. Individual clinical signs in female rats in acute inhalation toxicity study

CLINICAL SIGNS INDIVIDUAL DATA		
GT13-00173		SEX : FEMALE
Animal No.	OBSERVATIONS	TIME OCCURED
21	Normal	0[a] - 14 Day
	Terminal sacrifice	14 Day
22	Normal	0 - 14 Day
	Terminal sacrifice	14 Day
23	Normal	0 - 14 Day
	Terminal sacrifice	14 Day
24	Normal	0 - 14 Day
	Terminal sacrifice	14 Day
25	Normal	0 - 14 Day
	Terminal sacrifice	14 Day
26	Normal	0 - 14 Day
	Terminal sacrifice	14 Day
27	Normal	0 - 14 Day
	Terminal sacrifice	14 Day
28	Normal	0 - 14 Day
	Terminal sacrifice	14 Day
29	Normal	0 - 14 Day
	Terminal sacrifice	14 Day
30	Normal	0 - 14 Day
	Terminal sacrifice	14 Day
31	Normal	0 - 14 Day
	Terminal sacrifice	14 Day
32	Normal	0 - 14 Day
	Terminal sacrifice	14 Day
33	Normal	0 - 14 Day
	Terminal sacrifice	14 Day
34	Normal	0 - 14 Day
	Terminal sacrifice	14 Day
35	Normal	0 - 14 Day
	Terminal sacrifice	14 Day
36	Normal	0 - 14 Day
	Terminal sacrifice	14 Day
37	Normal	0 - 14 Day
	Terminal sacrifice	14 Day
38	Normal	0 - 14 Day
	Terminal sacrifice	14 Day
39	Normal	0 - 14 Day
	Terminal sacrifice	14 Day
40	Normal	0 - 14 Day
	Terminal sacrifice	14 Day

a : after exposure

Appendix 12. Individual body weights in male rats in acute inhalation toxicity study

INDIVIDUAL BODY WEIGHTS (Grams)

STUDY : GT13-00173 GROUP : CONTROL SEX : MALE

ANIMAL	0 DAY	1 DAY	3 DAY	7 DAY	14 DAY
1	136.55	151.07	161.61	184.91	214.79
2	146.64	158.09	166.61	184.94	212.29
3	146.66	155.58	165.77	184.55	213.07
4	153.55	164.95	176.84	196.54	223.35
5	157.19	167.05	175.82	195.93	222.62
N	5	5	5	5	5

GROUP : LOW

ANIMAL	0 DAY	1 DAY	3 DAY	7 DAY	14 DAY
6	139.53	153.65	165.36	187.28	217.57
7	146.20	158.18	166.22	187.39	219.09
8	147.07	158.00	165.08	188.47	212.99
9	153.16	161.35	170.70	188.48	217.60
10	159.72	166.25	175.13	195.21	216.38
N	5	5	5	5	5

GROUP : MEDIUM

ANIMAL	0 DAY	1 DAY	3 DAY	7 DAY	14 DAY
11	140.20	150.51	160.18	185.31	219.19
12	145.38	152.57	159.94	180.39	204.30
13	147.19	161.99	171.20	191.99	222.89
14	151.84	168.38	179.45	199.81	234.62
15	160.75	170.15	177.79	197.09	227.96
N	5	5	5	5	5

GROUP : HIGH

ANIMAL	0 DAY	1 DAY	3 DAY	7 DAY	14 DAY
16	141.50	155.11	161.54	180.22	209.73
17	144.72	153.06	158.49	177.86	203.18
18	148.17	155.79	164.39	182.01	210.51
19	149.45	155.84	164.47	180.73	203.97
20	162.74	171.69	178.46	196.75	217.03
N	5	5	5	5	5

Appendix 13. Individual body weights in female rats in acute inhalation toxicity study

INDIVIDUAL BODY WEIGHTS (Grams)

STUDY : GT13-00173 GROUP : CONTROL SEX : FEMALE

ANIMAL	0 DAY	1 DAY	3 DAY	7 DAY	14 DAY
21	113.29	118.05	124.30	134.36	139.88
22	125.98	127.93	133.79	142.35	152.22
23	126.21	129.66	133.11	141.17	151.66
24	131.04	135.11	138.19	149.05	158.56
25	132.55	136.55	140.77	151.45	159.82
N	5	5	5	5	5

GROUP : LOW

ANIMAL	0 DAY	1 DAY	3 DAY	7 DAY	14 DAY
26	116.91	119.30	123.67	132.78	141.58
27	125.63	128.58	133.48	141.65	151.32
28	126.79	132.41	138.01	142.51	153.71
29	130.99	136.74	140.55	149.44	158.95
30	134.20	138.80	142.72	150.13	157.83
N	5	5	5	5	5

GROUP : MEDIUM

ANIMAL	0 DAY	1 DAY	3 DAY	7 DAY	14 DAY
31	119.54	124.41	128.09	134.43	145.98
32	123.47	124.13	130.19	136.43	140.01
33	129.01	131.35	138.07	145.20	153.71
34	130.76	135.07	137.65	146.73	156.51
35	134.41	136.45	141.61	149.47	160.86
N	5	5	5	5	5

GROUP : HIGH

ANIMAL	0 DAY	1 DAY	3 DAY	7 DAY	14 DAY
36	121.62	126.88	131.14	140.87	150.67
37	122.70	127.91	133.08	140.17	146.57
38	129.71	132.98	137.02	146.61	151.88
39	130.61	134.70	136.74	143.84	150.94
40	135.19	138.77	140.79	150.51	156.09
N	5	5	5	5	5

Appendix 14. Individual gross findings in male rats

INDIVIDUAL GROSS FINDINGS

STUDY : GT13-00173 GROUP : Control SEX : MALE

ANIMAL	FATE	DAY	LOCATION	OBSERVATION
1	Terminal sacrifice	14		normal
2	Terminal sacrifice	14		normal
3	Terminal sacrifice	14		normal
4	Terminal sacrifice	14		normal
5	Terminal sacrifice	14		normal

STUDY : GT13-00173 GROUP : Low SEX : MALE

ANIMAL	FATE	DAY	LOCATION	OBSERVATION
6	Terminal sacrifice	14		normal
7	Terminal sacrifice	14		normal
8	Terminal sacrifice	14		normal
9	Terminal sacrifice	14		normal
10	Terminal sacrifice	14		normal

STUDY : GT13-00173 GROUP : Middle SEX : MALE

ANIMAL	FATE	DAY	LOCATION	OBSERVATION
11	Terminal sacrifice	14		normal
12	Terminal sacrifice	14		normal
13	Terminal sacrifice	14		normal
14	Terminal sacrifice	14		normal
15	Terminal sacrifice	14		normal

STUDY : GT13-00173 GROUP : High SEX : MALE

ANIMAL	FATE	DAY	LOCATION	OBSERVATION
16	Terminal sacrifice	14		normal
17	Terminal sacrifice	14		normal
18	Terminal sacrifice	14		normal
19	Terminal sacrifice	14		normal
20	Terminal sacrifice	14		normal

Appendix 15. Individual gross findings in female rats in acute inhalation toxicity study

INDIVIDUAL GROSS FINDINGS

STUDY : GT13-00173 GROUP : Control SEX : FEMALE

ANIMAL	FATE	DAY	LOCATION	OBSERVATION
21	Terminal sacrifice	14		normal
22	Terminal sacrifice	14		normal
23	Terminal sacrifice	14		normal
24	Terminal sacrifice	14		normal
25	Terminal sacrifice	14		normal

STUDY : GT13-00173 GROUP : Low SEX : FEMALE

ANIMAL	FATE	DAY	LOCATION	OBSERVATION
26	Terminal sacrifice	14		normal
27	Terminal sacrifice	14		normal
28	Terminal sacrifice	14		normal
29	Terminal sacrifice	14		normal
30	Terminal sacrifice	14		normal

STUDY : GT13-00173 GROUP : Middle SEX : FEMALE

ANIMAL	FATE	DAY	LOCATION	OBSERVATION
31	Terminal sacrifice	14		normal
32	Terminal sacrifice	14		normal
33	Terminal sacrifice	14		normal
34	Terminal sacrifice	14		normal
35	Terminal sacrifice	14		normal

STUDY : GT13-00173 GROUP : High SEX : FEMALE

ANIMAL	FATE	DAY	LOCATION	OBSERVATION
36	Terminal sacrifice	14		normal
37	Terminal sacrifice	14		normal
38	Terminal sacrifice	14		normal
39	Terminal sacrifice	14		normal
40	Terminal sacrifice	14		normal

Appendix 16. Study plan amendment sheet

시험계획서 (변경 · 이탈)기록서

시험제목	Fisher 344 랫드를 이용한 MWCNT의 급성흡입독성시험		
시험기간	2013.04.19.~2013.05.31.	시험번호	GT13-00173
(변경) · 이탈 사 항		(변경) · 이탈 사 유	
※ 시험일정 · 변경 전 동물 입수예정일 : 2013 년 04 월 25 일 시험물질 노출일 : 2013 년 04 월 30 일 부검일 : 2013 년 05 월 14 일 최종보고서(안) 제출예정일 : 2013 년 05 월 31 일 · 변경 후 동물 입수예정일 : 2013 년 04 월 25 일 시험물질 노출일 : 2013 년 04 월 30 일 부검일 : 2013 년 05 월 14 일 최종보고서(안) 제출예정일 : 2013 년 06 월 21 일		· 의뢰한 Transmission Electron Microscope (TEM) 분석 지연으로 인한 최종보고서(안) 제출일 변경	
시험책임자 : 최병노 (서명)		운영책임자 : 이진기 (서명)	
날 짜 : 2013 년 05 월 22 일		날 짜 : 2013 년 05 월 22 일	
신뢰성보증부서 : 송경석 (인)		날짜 : 2013 년 05 월 22 일	
시험의뢰자 : (인)		날짜 : 년 월 일	

Appendix 16. Study plan amendment sheet (continued)

시험계획서 (변경 · 이탈)기록서

시험제목	Fisher 344 랫드를 이용한 MWCNT의 급성흡입독성시험		
시험기간	2013.04.19.~2013.05.31.	시험번호	GT13-00173
변경 · 이탈 사 항		변경 · 이탈 사 유	
※ 시험일정 - 변경 전 동물 입수예정일 : 2013 년 04 월 25 일 시험물질 노출일 : 2013 년 04 월 30 일 부검일 : 2013 년 05 월 14 일 최종보고서(안) 제출예정일 : 2013 년 06 월 21 일 - 변경 후 동물 입수예정일 : 2013 년 04 월 25 일 시험물질 노출일 : 2013 년 04 월 30 일 부검일 : 2013 년 05 월 14 일 최종보고서(안) 제출예정일 : 2013 년 07 월 05 일		· 의뢰한 Transmission Electron Microscope (TEM) 분석 지연으로 인한 최종보고서(안) 제출일 변경	
시험책임자 :		운영책임자 :	
날 짜 : 2013 년 06 월 13 일		날 짜 : 2013 년 06 월 13 일	
신뢰성보증부서 :		날짜 : 2013 년 06 월 13 일	
시험의뢰자 : (인)		날짜 : 년 월 일	

Appendix 17. KCL GLP certification of NIER

지정번호 (Certification No.)		화학물질 유해성 시험기관 지정서 GLP Certificate
제2008-4호		
①	시험기관	한국생활환경시험연구원 안전성평가본부
	Test Facility Name	Korea Environment and Merchandise Testing Institute Bio-Safety Evaluation Headquarters
②	소 재 지	인천광역시 연수구 송도동 7-44
	Address	7-44, Songdo-Dong, Yeonsu-Gu, Incheon, 406-840, Korea
③	대 표 자	김 창 로
	President	Chang-Ro Kim
④	운영책임자	유 일 재
	Test Facility Management	Il-Je Yu
⑤	시험의 범위	- 급성경구독성시험, 유전독성시험(복귀돌연변이시험, 염색체이상시험, 소핵시험). (유효기간 : 2006년 3월 31일부터). 끝. - 급성피부자극성 및 부식성시험, 급성안자극성 및 부식성시험, 급성흡입독성시험. (유효기간 : 2007년 4월 17일부터). 끝. - 아급성독성시험, 피부감작성시험. (유효기간 : 2008년 8월 25일부터). 끝.
	Test Scope	- Acute oral toxicity, Genetic Toxicity(Ames test, Chromosome abberation test, Micronucleus test) (Validation : since Mar. 31, 2006). - Acute dermal irritation/corrosion, Acute eye irritation/ corrosion, Acute inhalation toxicity (Validation : since Apr. 17, 2007). - Subchronic toxicity, Skin sensitization (Validation : since Aug. 25, 2008).

「유해화학물질관리법」 제14조, 같은 법 시행령 제12조 및 같은 법 시행규칙 제10조제2항에 따라 화학물질 유해성 시험기관(GLP시험기관)으로 지정합니다.

It is hereby certified that the test facility was inspected by the national compliance monitoring authority regarding compliance with the Principles of Good Laboratory Practice.

Issue date 2008년(year) 8월(month) 25일(date)

국립환경과학원장 ㊞

President, National Institute of Environmental Research

(뒤 쪽)-1

<변경사항>

일자	내용	확인
2009. 5. 20	운영책임자 변경 : 유 일 재 (Il-Je Yu) 에서 송 경 석 (Kyung-Seuk Song) 으로 변경	
2009. 11. 16 (국문)	시험의 범위 : 급성경피독성시험, 어류급성독성시험 (유효기간 : 2009년 11월 16일 부터) 끝.	
〃 (영문)	Test Scope : Acute dermal toxicity, Fish: acute toxicity (Validation : since Nov. 16, 2009).	
2010. 8. 2	대표자 변경 : 김 창 로 (Chang-ro Kim) 에서 오 태 식 (Taeshik Oh) 로 변경	GLP 확인
2010. 8. 2	기관명 변경 : "한국건설생활환경시험연구원 바이오융합본부" 로 변경 *영문명 (Bioconvergence Technology Division, Korea Conformity Laboratories)	GLP 확인
2011. 9. 9	운영책임자 변경 : 송 경 석 (Kyung-Seuk Song) 에서 이 진 규 (Jin Kyu Lee) 으로 변경	GLP 확인

<처분사항>

일자	내용	확인

<참고사항>

일자	내용	확인
2010. 12.	정기사후평가 결과, GLP 규정을 준수하고 있음 (GLP Compliance)	GLP 확인
2012. 7. 2	정기사후평가 결과, GLP 규정을 준수하고 있음 (GLP Compliance)	GLP 확인

Appendix 17. KCL GLP certification of NIER (continued)

화학물질유해성시험기관 지정서
제2008-4호

(뒤 쪽)-2

<변경사항>

일자	내용	확인
2011. 9. 9	기관명변경: "한국건설생활환경시험연구원 바이오융합단"으로 변경 (Bioconvergence Technology Department, Korea Conformity Laboratories)	GLP 확인
2011. 11. 3	대표자 변경 : 오태식 (Taeshik Oh)에서 송 재빈 (Jae Bin Song)으로 변경	GLP 확인
2012. 7. 2	기관명변경 : "한국건설생활환경시험연구원 바이오융합단"으로 변경 (Bioconvergence Technology Laboratory, Korea Conformity Laboratories)	GLP 확인
2012. 7. 2	시험의 범위 : 물벼룩 급성독성시험, 조류생장저해시험 [Test Scope : Daphnia sp. acute toxicity, Algae: growth inhibition (since July. 2, 2012.)]	GLP 확인

<처분사항>

일자	내용	확인

<참고사항>

일자	내용	확인

Appendix 18. Quality assurance statement-Original

신 뢰 성 보 증 확 인 서

시험번호 : GT13-00173

시 험 명 : Fisher 344 랫드를 이용한 MWCNT의 급성흡입독성시험

이 보고서에 기술된 시험을 독립적으로 아래와 같이 시험과정 단계별로 점검하였으며 각 점검결과를 표준작업지침서에 따라 시험책임자와 운영책임자에게 통보 및 보고하였다.

본 시험은 국립환경과학원고시 제2013-02호(2013년 01월 09일)의 '화학물질 유해성 시험방법' 및 OECD Guidelines for the Testing of Chemical No. 403 'Acute Inhalation Toxicity'(Adopted 7th Sep, 2009)에 따라 수행되었으며, 보고서의 방법 및 결과의 기술이 시험의 실시과정에서 발생한 시험기초자료를 바탕으로 정확히 반영되었음을 확인하였다.

점 검 내 용	실 시 일	시험책임자에게 통보일	운영책임자에게 보고일
시험계획서 점검	2013. 04. 19	2013. 04. 19	2013. 04. 22
시험물질 및 대조물질	2013. 04. 25	2013. 04. 25	2013. 04. 25
동물입수	2013. 04. 25	2013. 04. 25	2013. 04. 25
시험물질조제	2013. 04. 30	2013. 04. 30	2013. 04. 30
동물사육 및 투여	2013. 04. 30	2013. 04. 30	2013. 04. 30
증상관찰 및 측정	2013. 05. 14	2013. 05. 14	2013. 05. 14
부검	2013. 05. 14	2013. 05. 14	2013. 05. 14
시험기초자료	2013. 08. 05	2013. 08. 05	2013. 08. 05
최종보고서 점검	2013. 08. 05	2013. 08. 05	2013. 08. 05

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신뢰성보증책임자 송경석 (인)

2013 년 08 월 06 일

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주 시험담당자	성재혁 주시험담당자	날짜	2013. 08. 01
시험물질 조제	성재혁 시험물질 조제분석 책임자	날짜	2013. 08. 01
동물관리	백민원 동물관리 책임자	날짜	2013.08.05
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자료보관	김효동 자료보관 책임자	날짜	2013.08.05